Figure 1

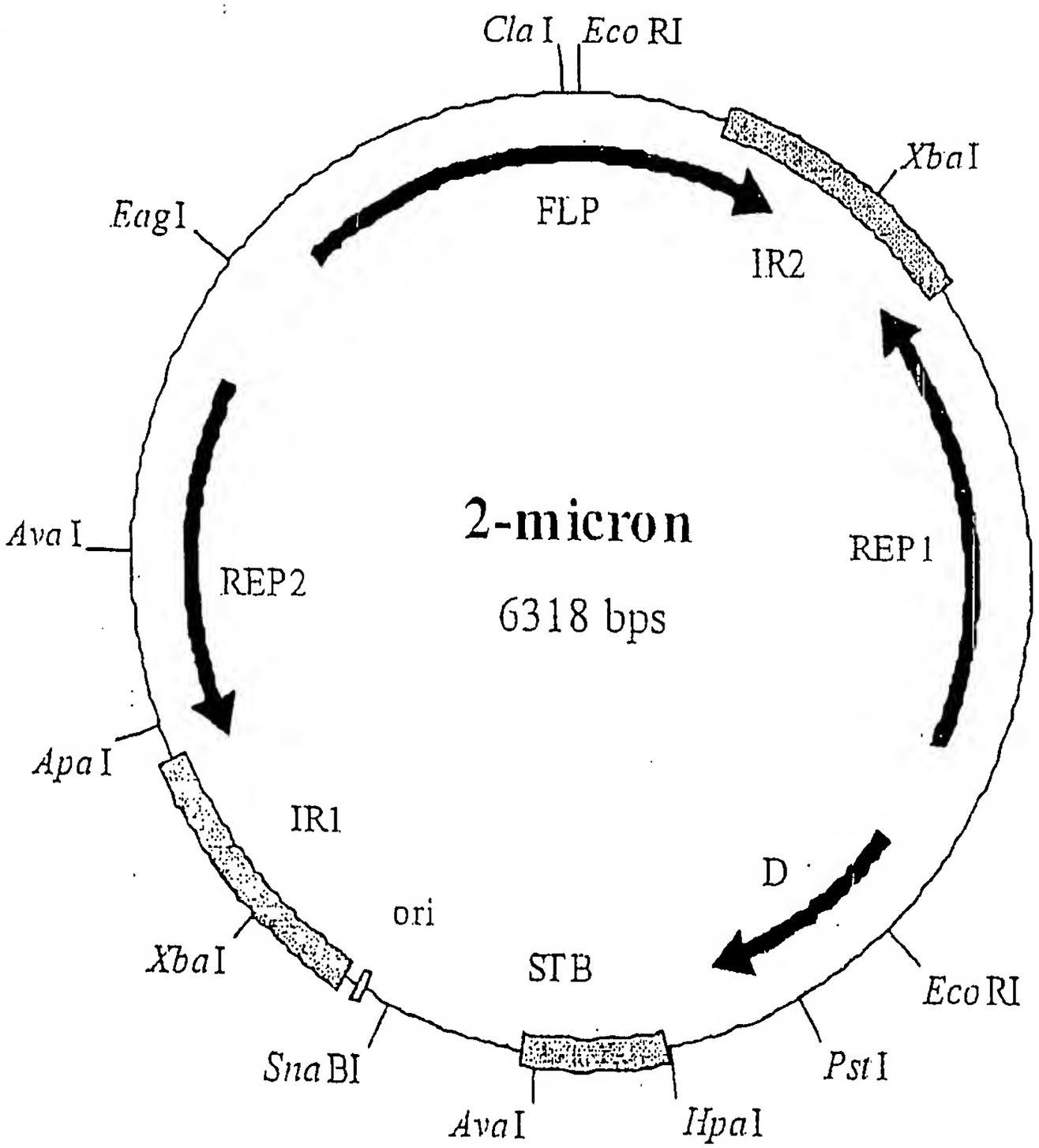
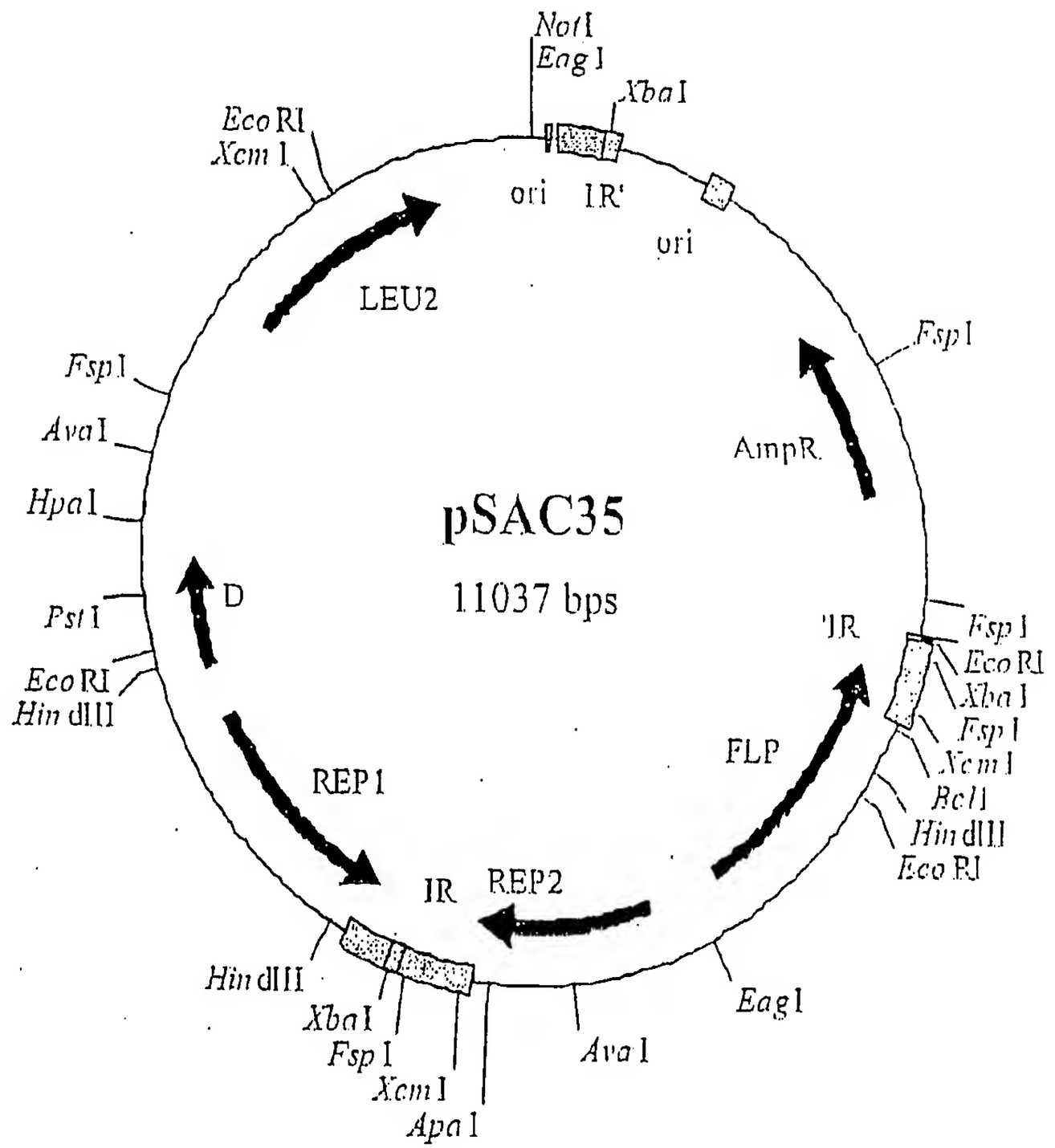


Figure 2



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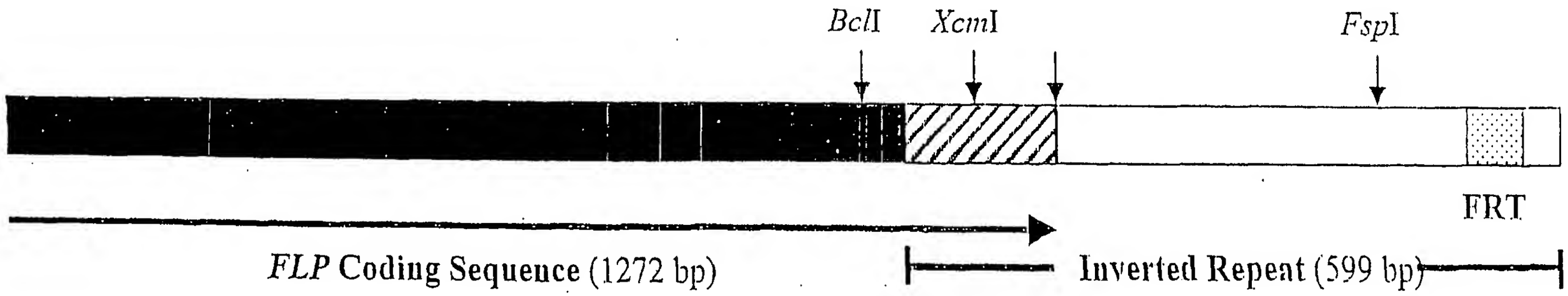
PCT/GB2004/005462

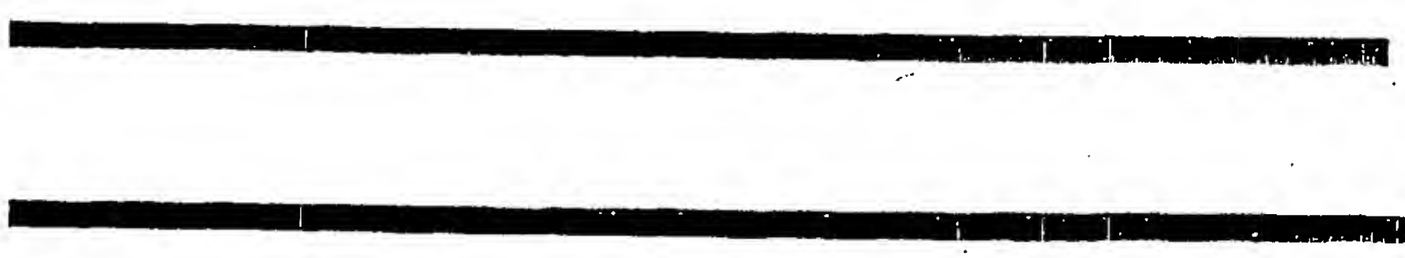
Figure 3

A) Restriction Endonuclease Sites used for DNA Insertions in *FLP* and the *FLP* Inverted



B) **Flp Protein** (423 amino acid residues)

C) **Truncated FLP Protein Products**



Flp (1-353) from termination at *Bcl*I-site

Flp (1-382) from termination at *Xcm*I-

Figure 4

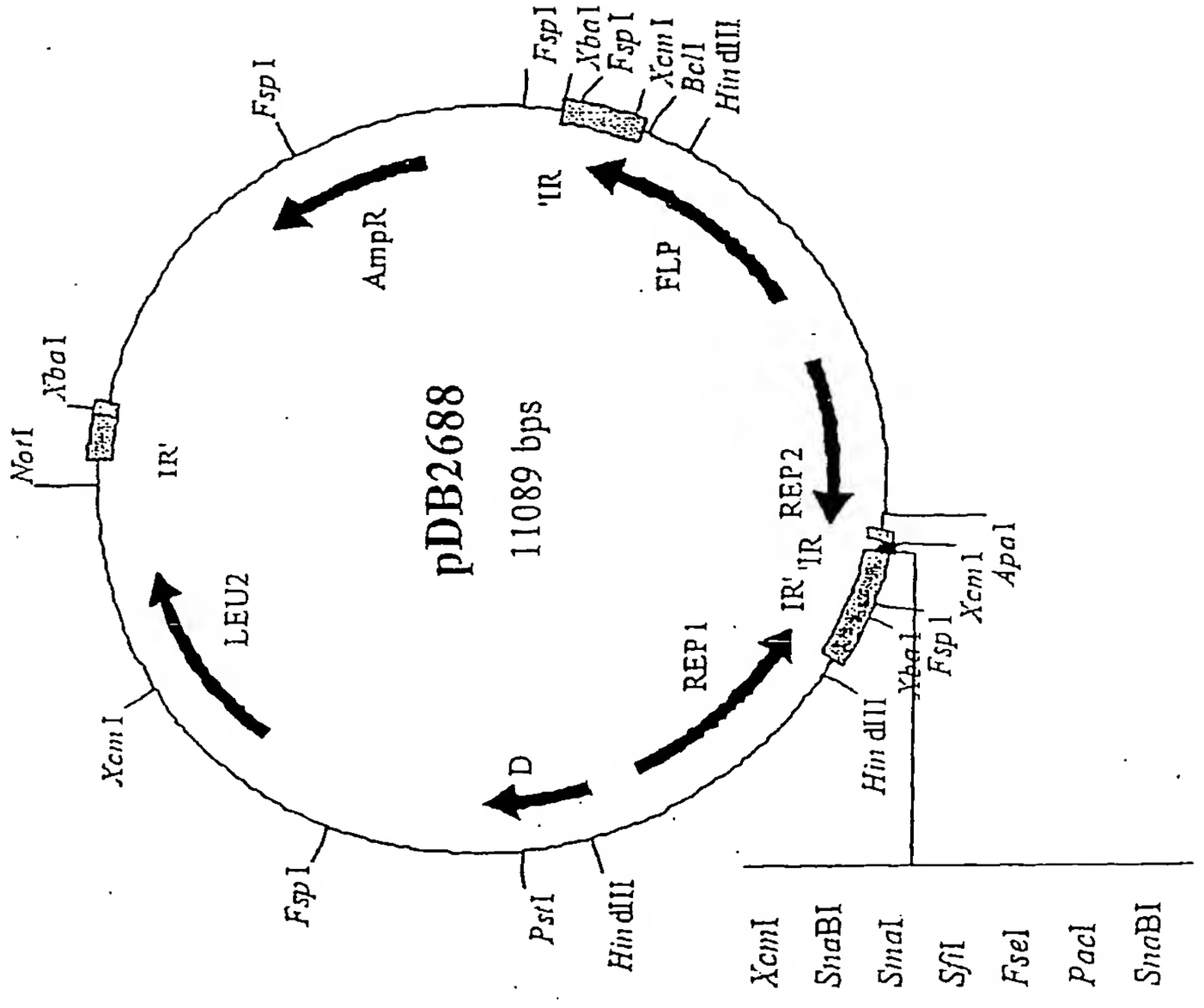


Figure 5

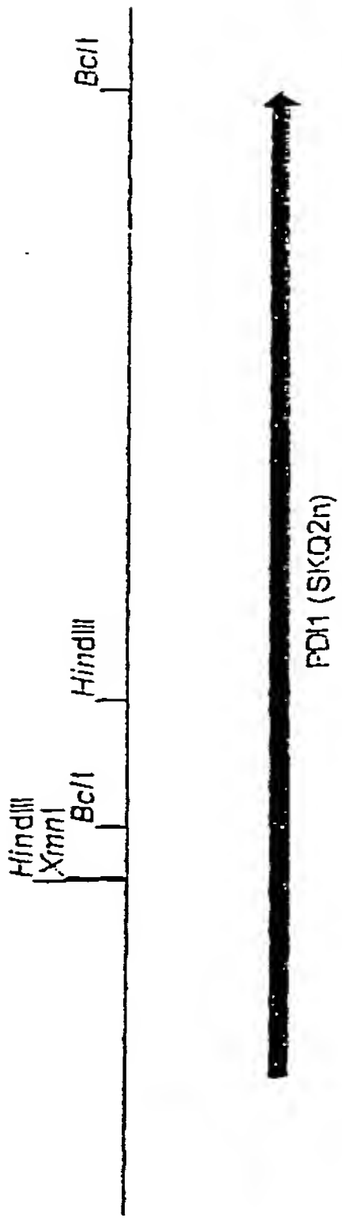


Figure 6

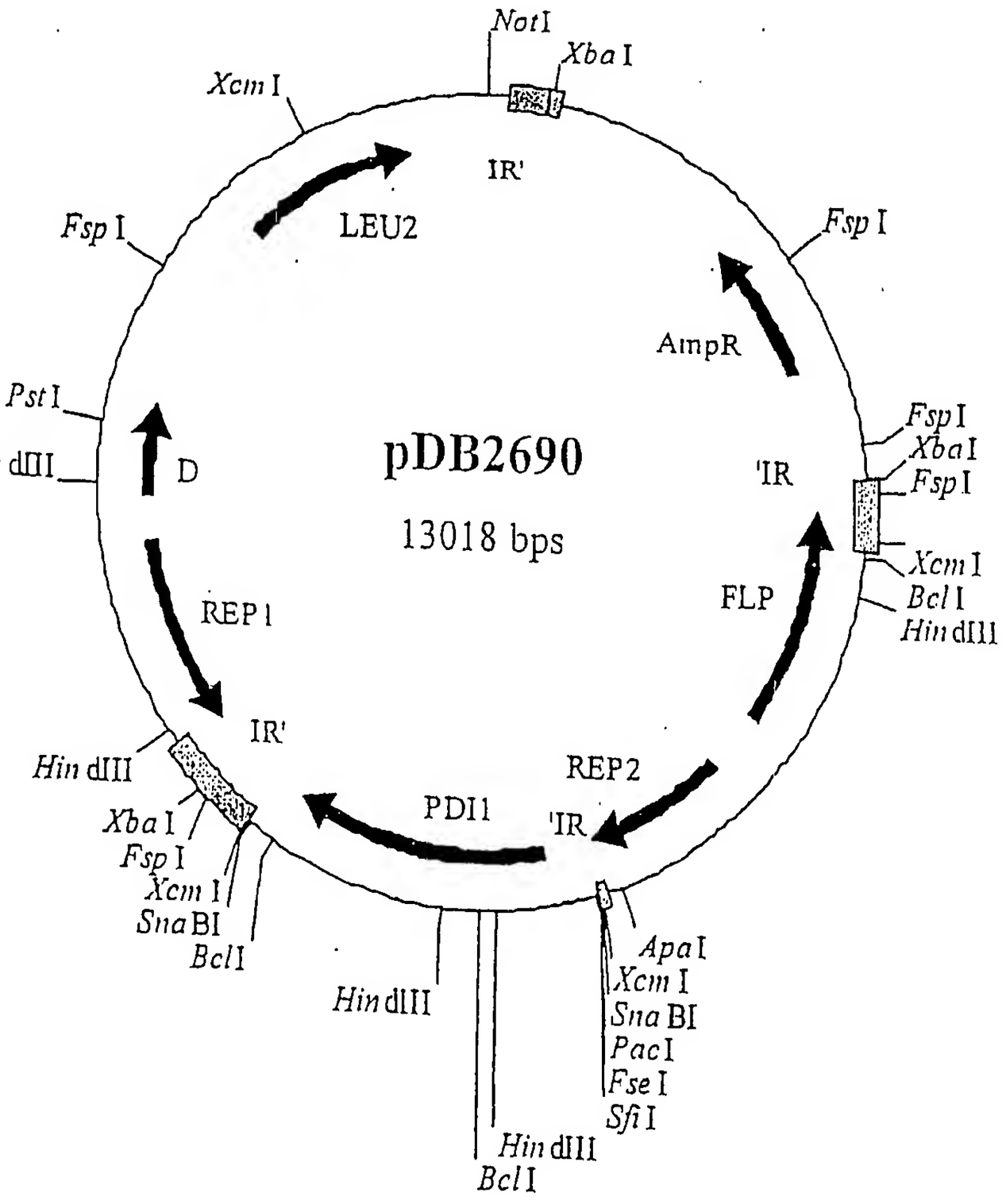
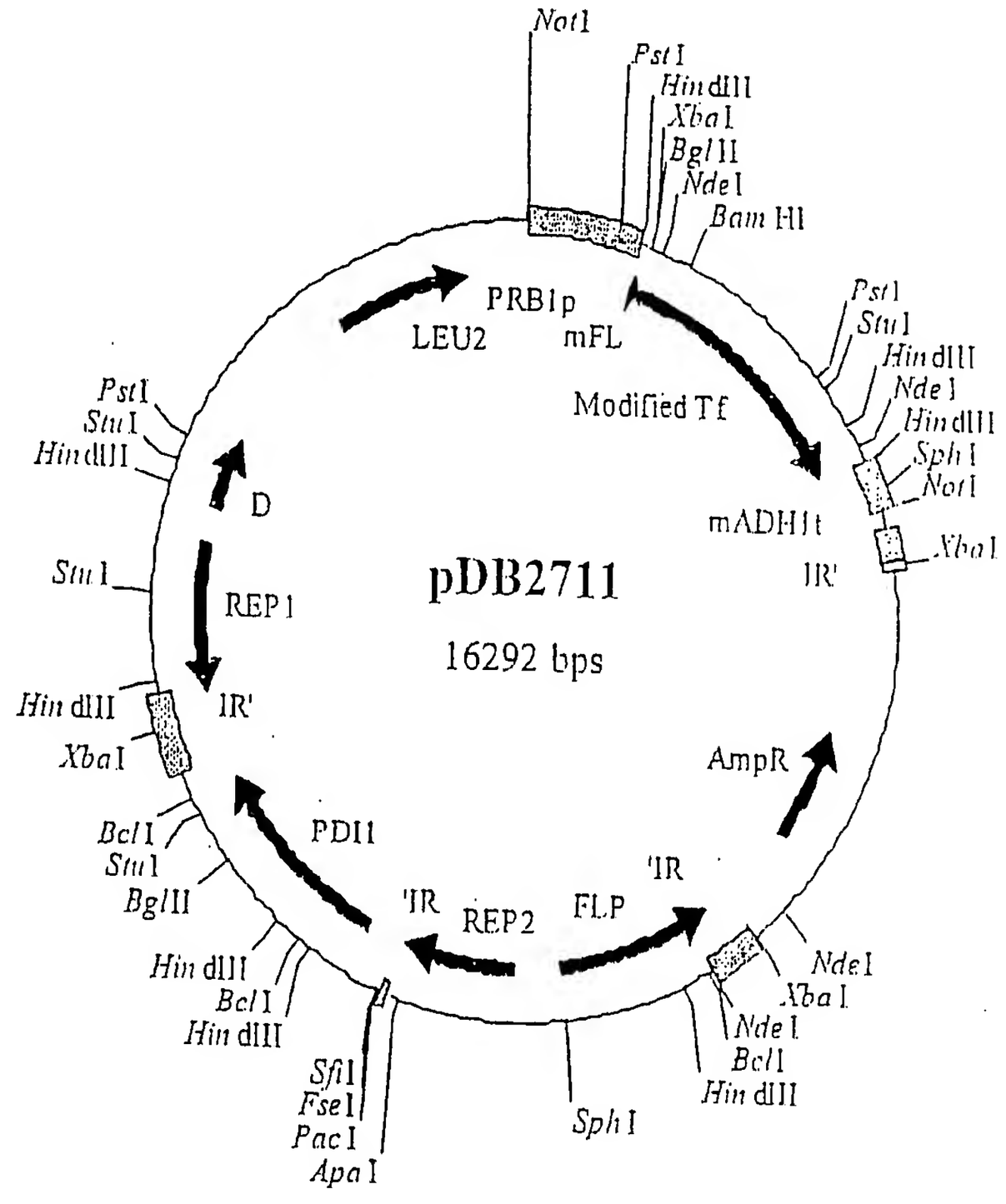


Figure 7

mFL = modified HSA(pre)/MFα1(pro) fusion leader sequence



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PCT/GB2004/005462

Figure 8

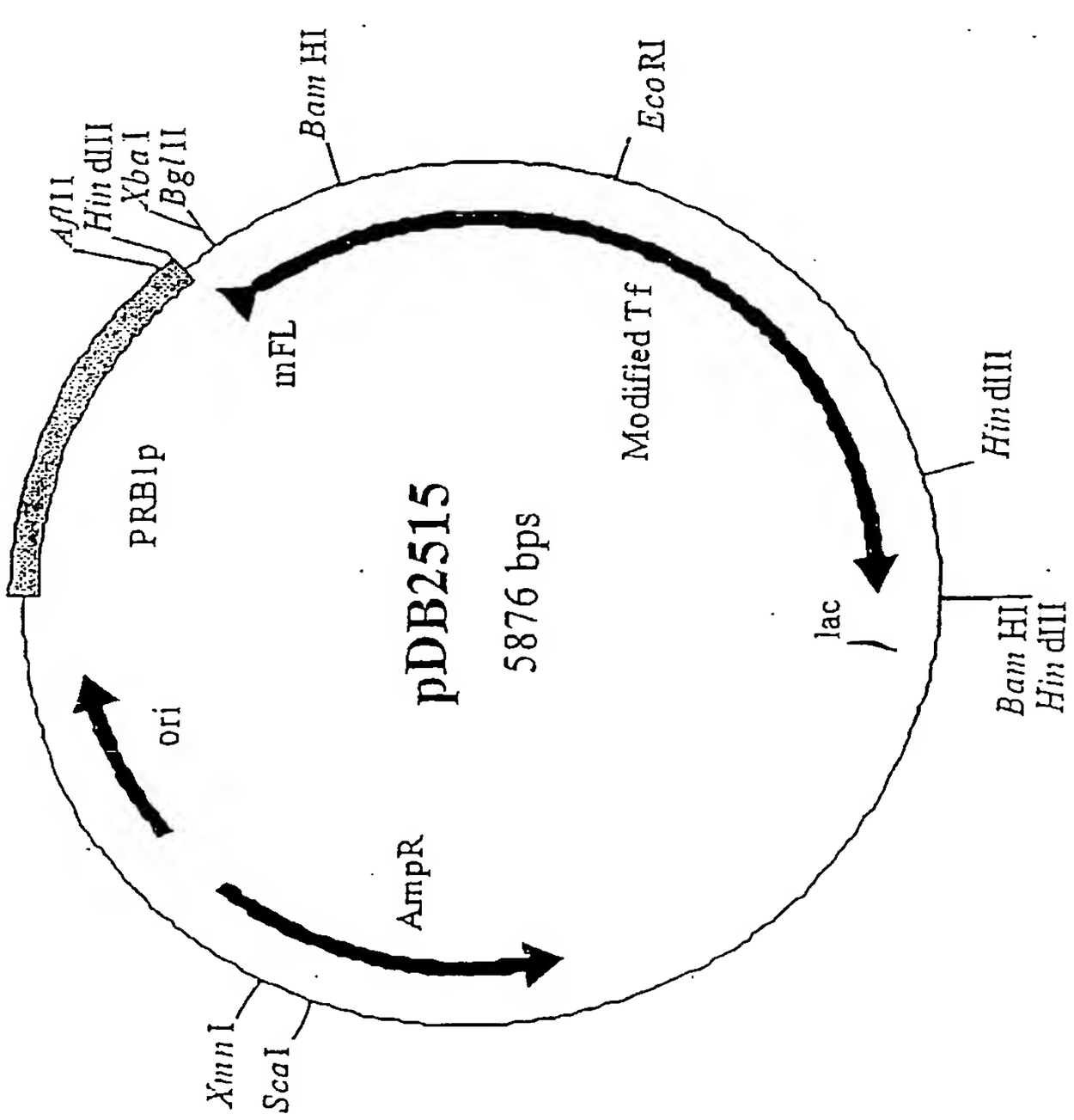
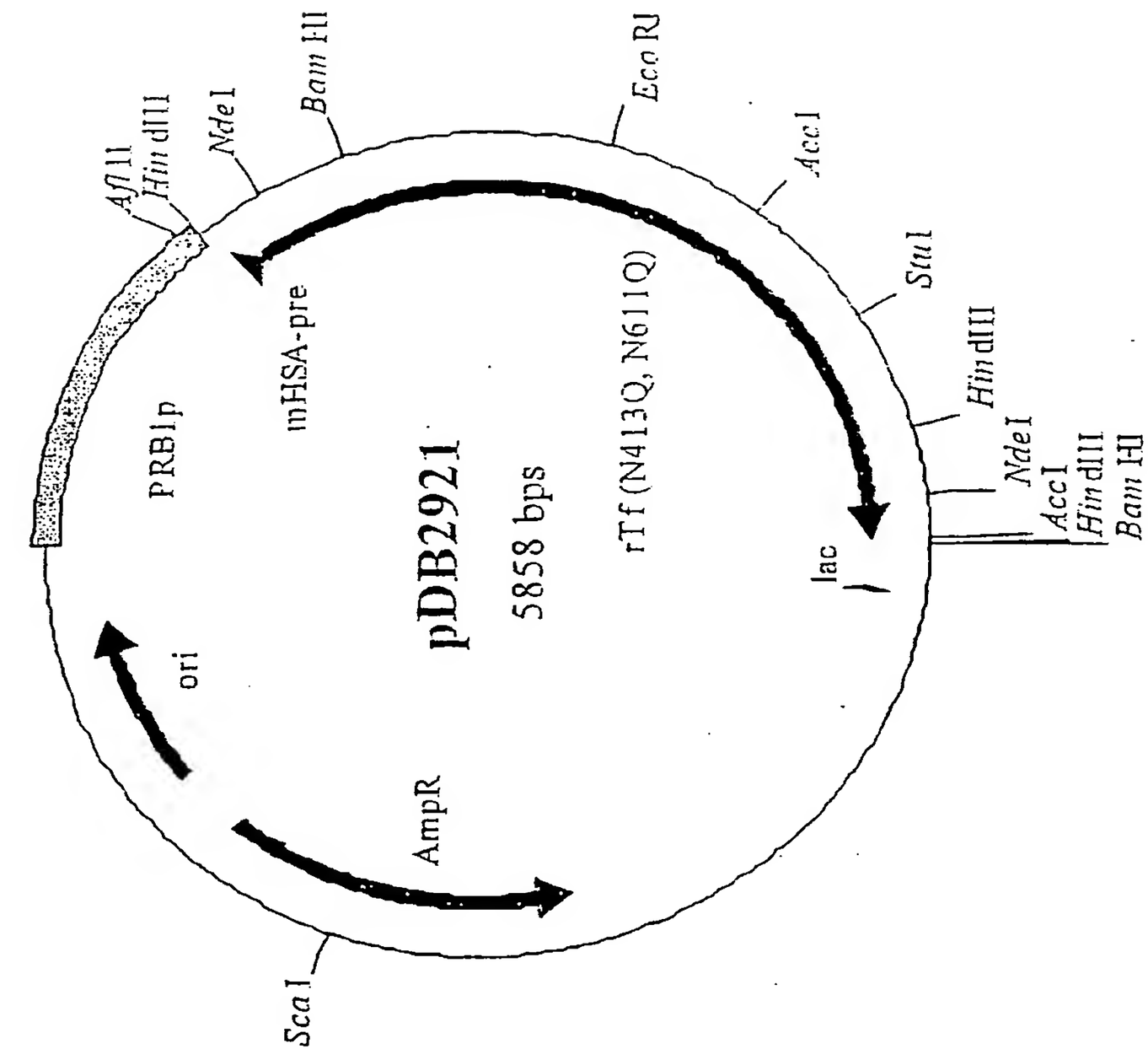


Figure 9



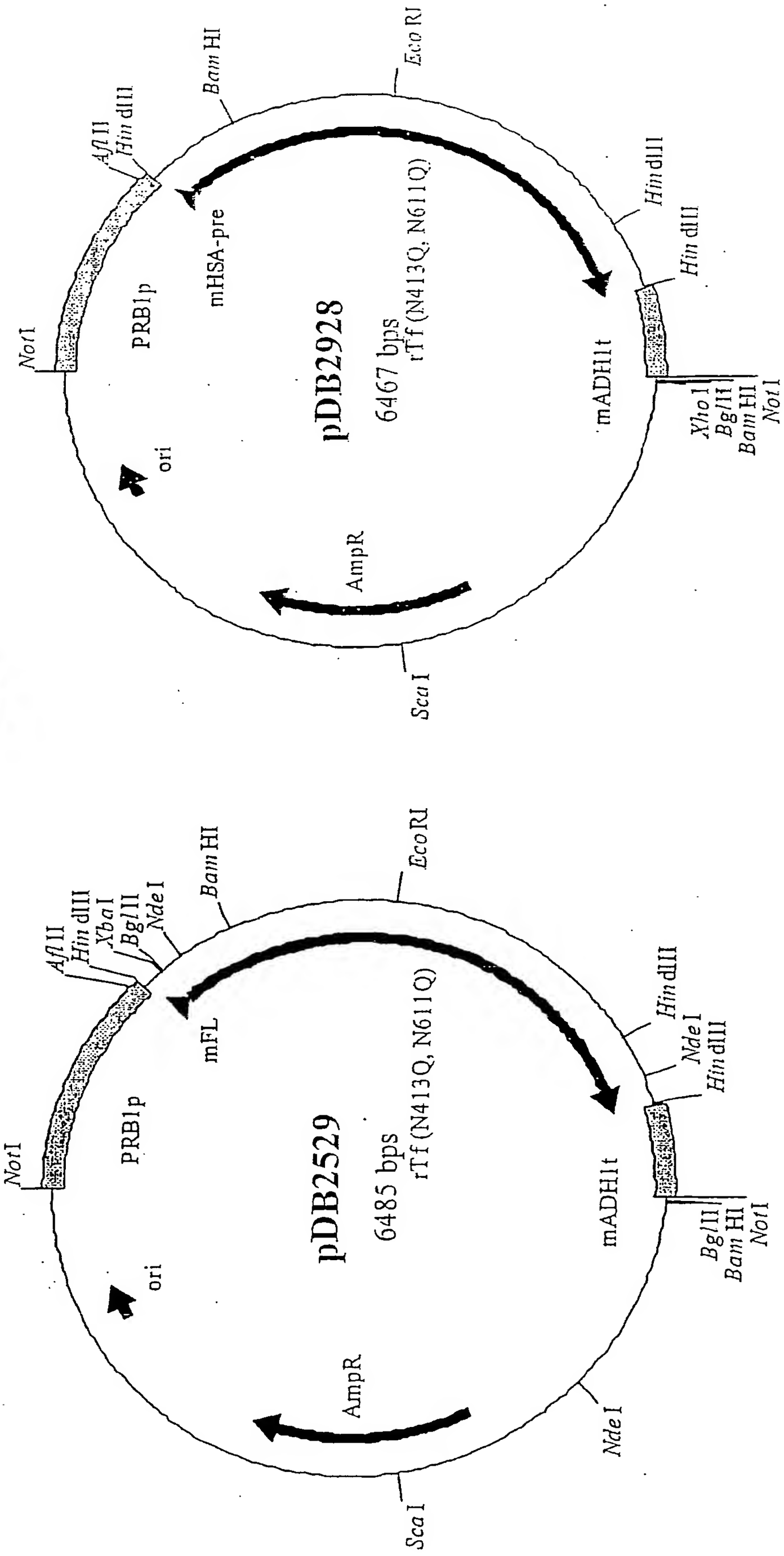


Figure 11

Figure 10

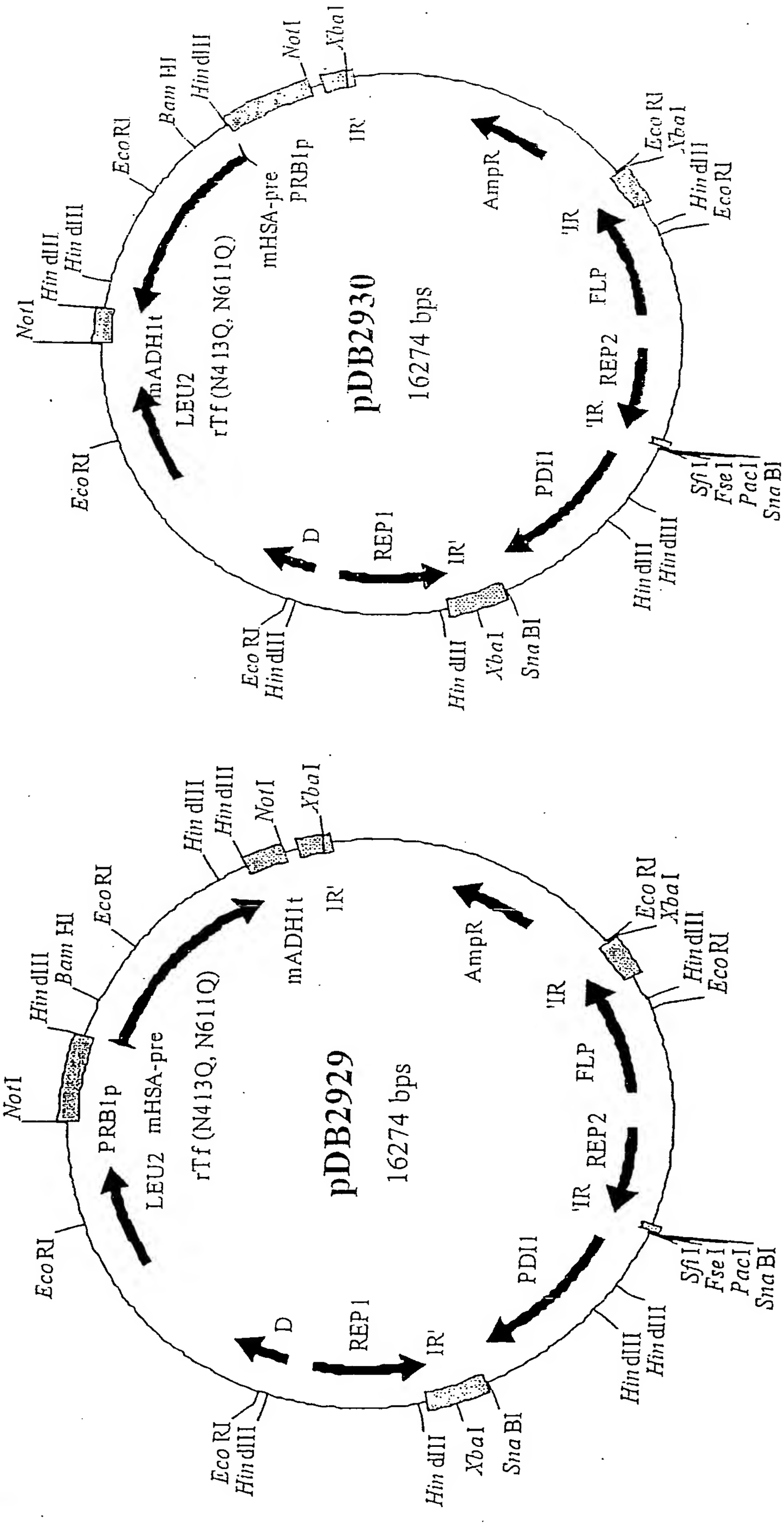


Figure 13

Figure 12

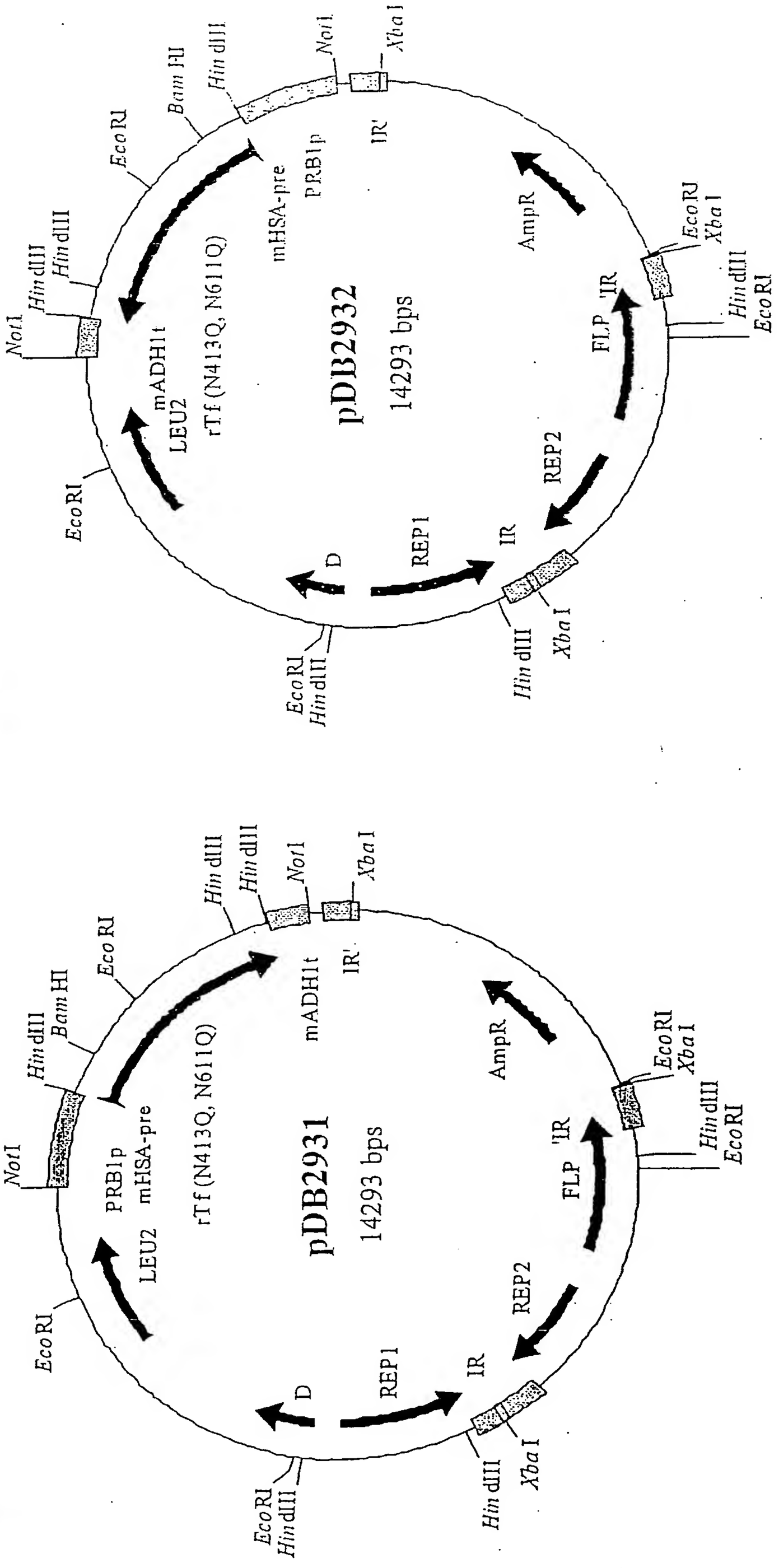


Figure 15

Figure 14

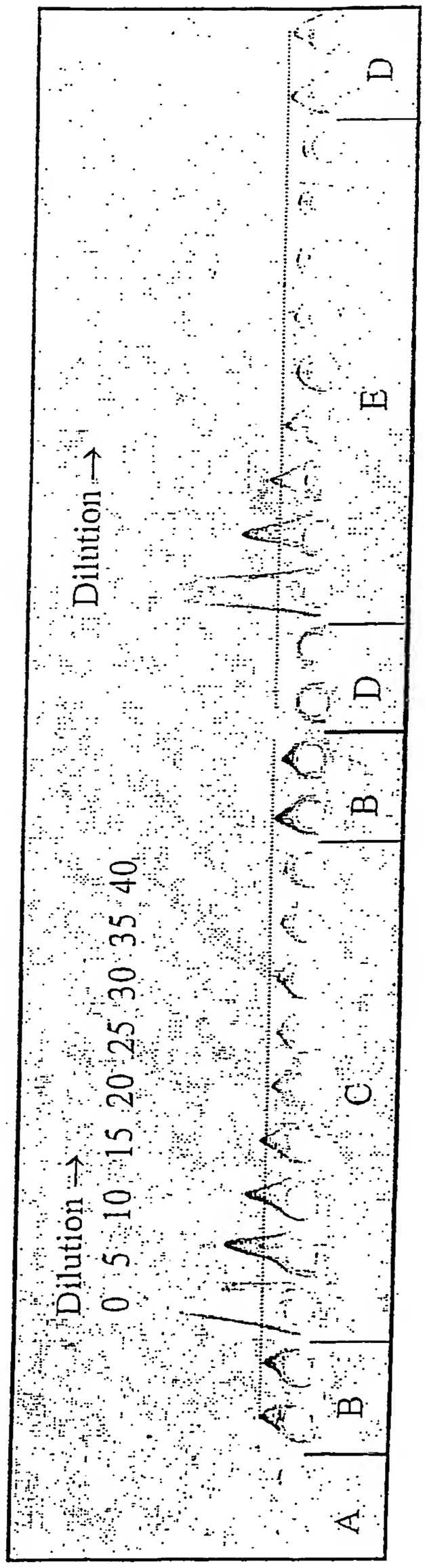


Figure 16

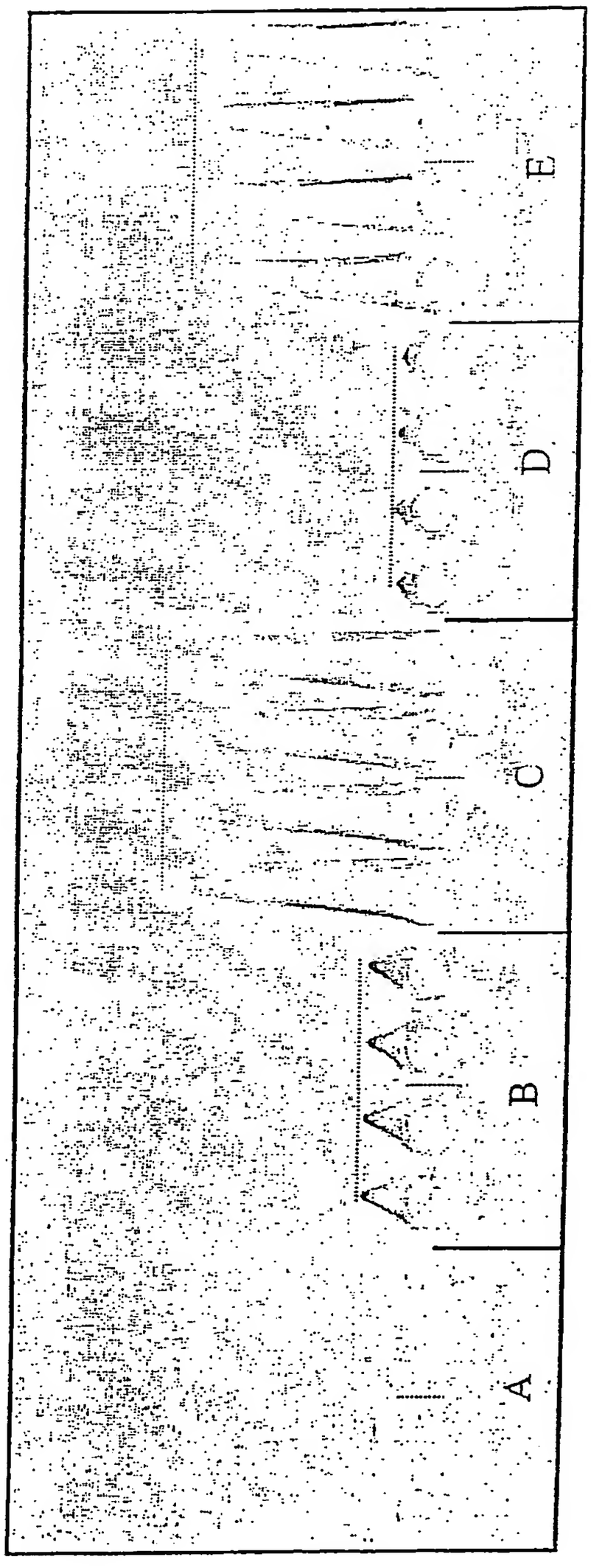


Figure 17

Figure 18

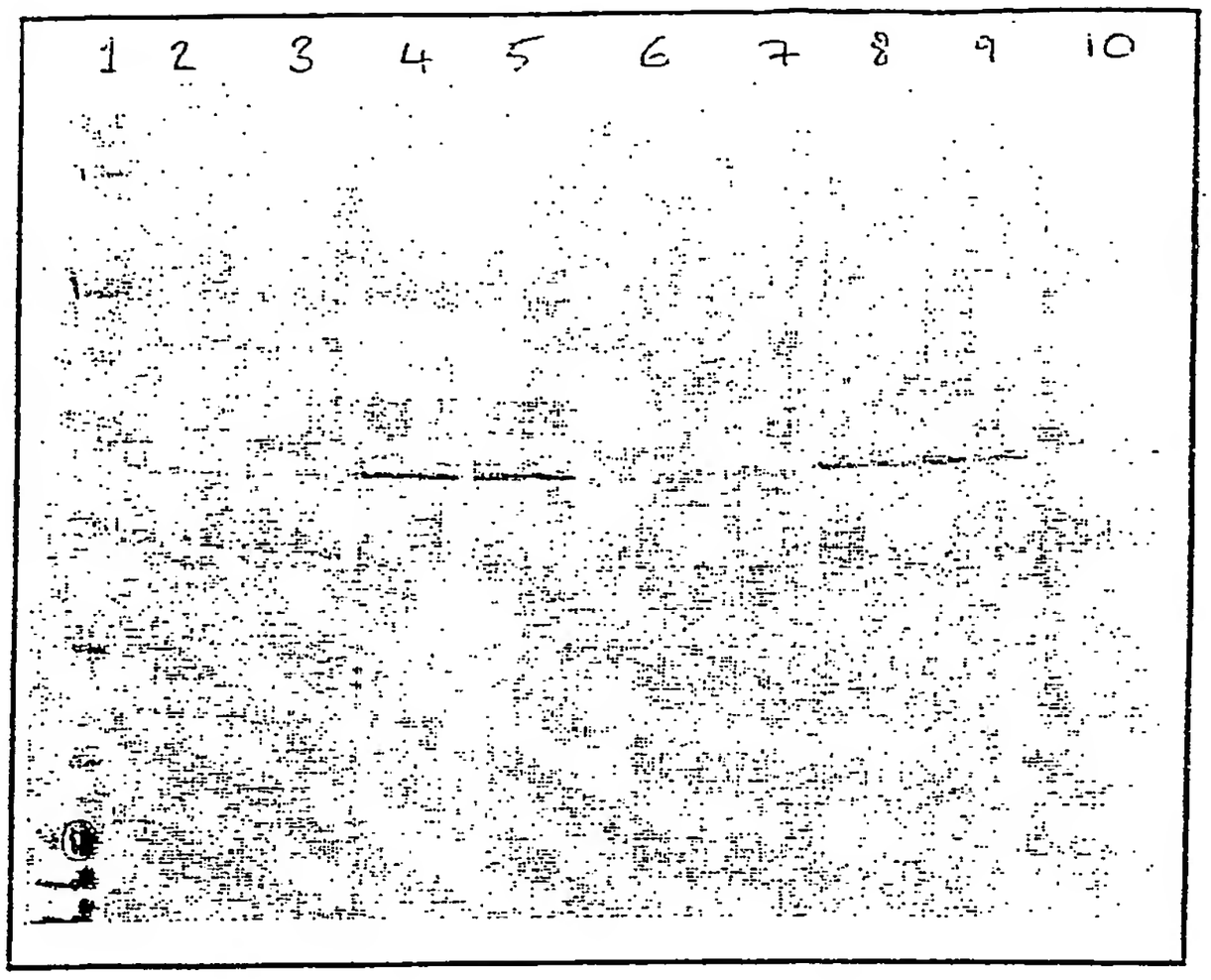
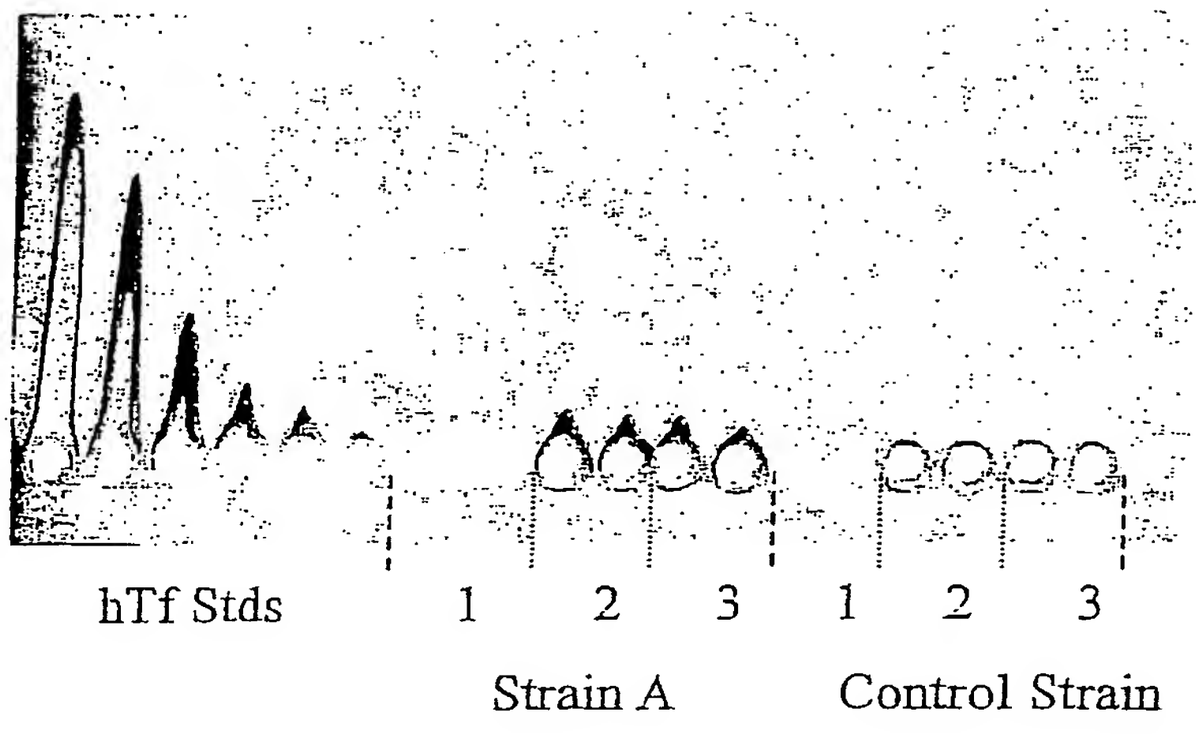


Figure 19



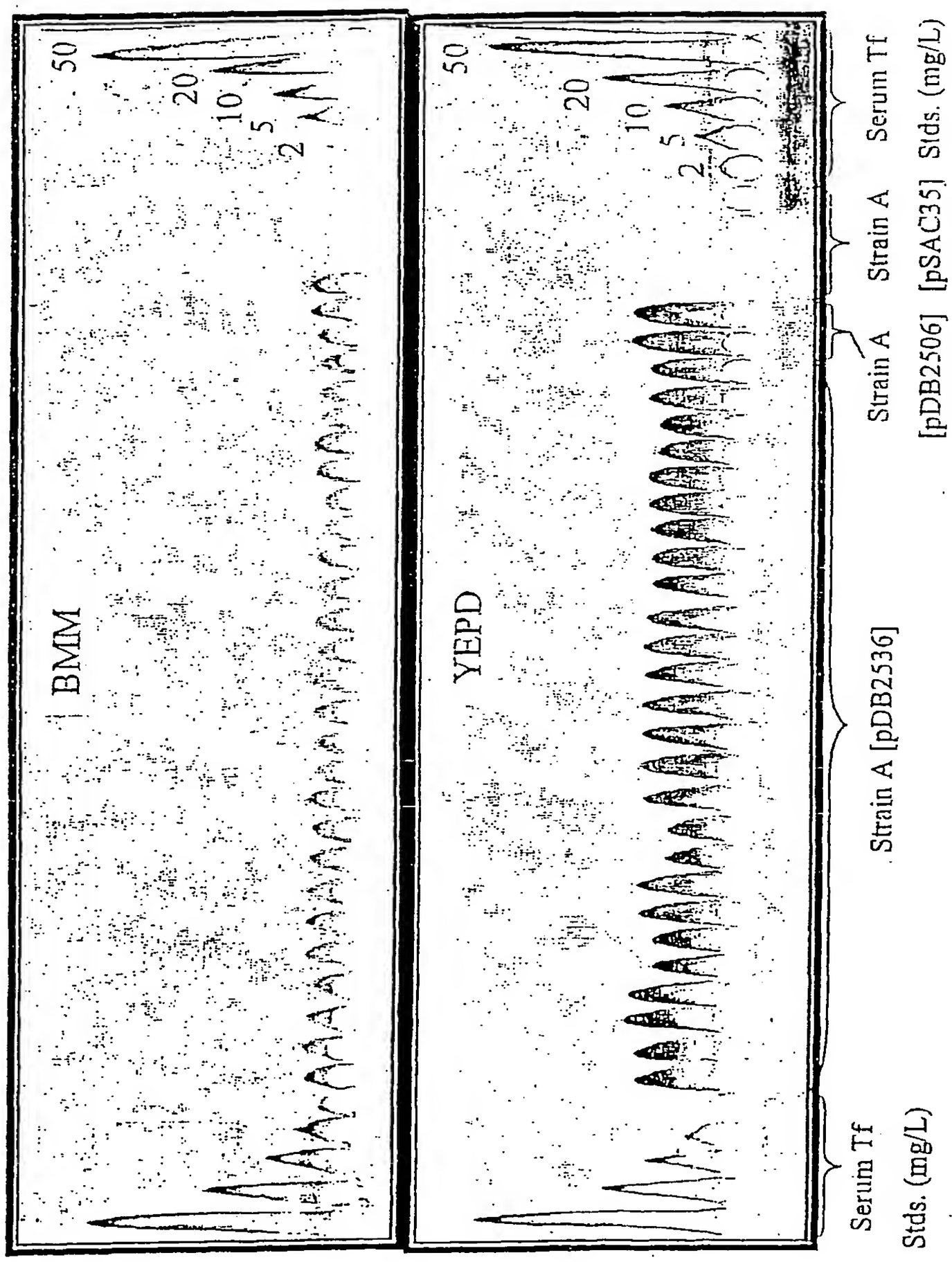

BMM
YEPD
50
20
10
5
2
1
Strain A [pDB2536]
Strain A [pDB2506]
Strain A [pSAC35]
Serum Tf
Stds. (mg/L)
(control)

Figure 20

Figure 21

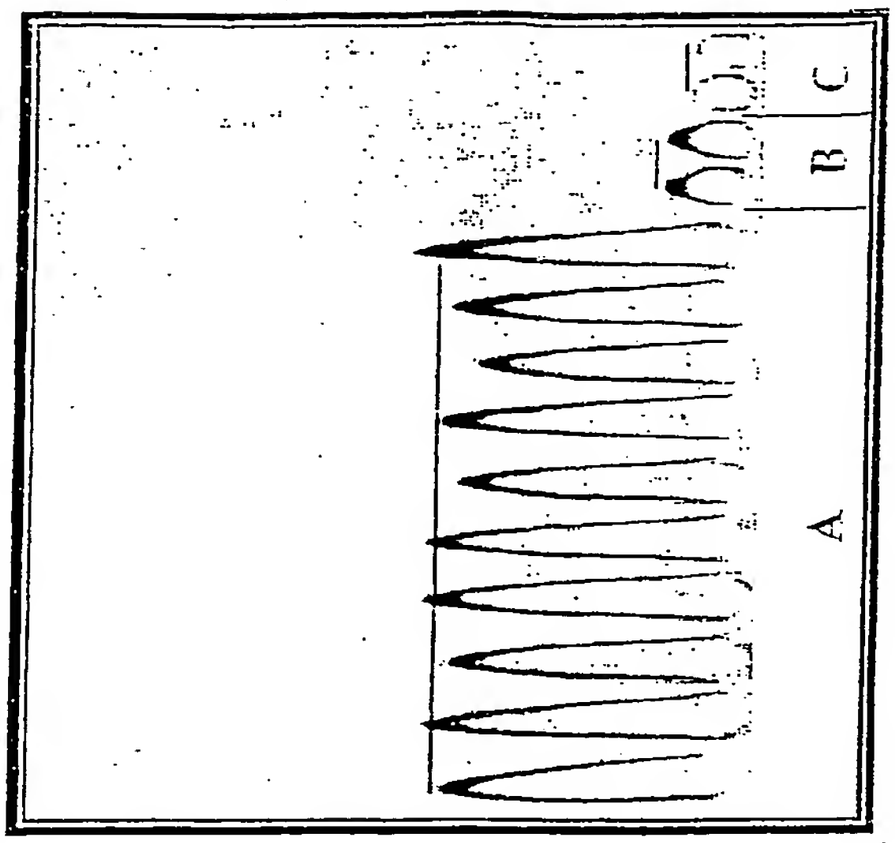


Figure 23

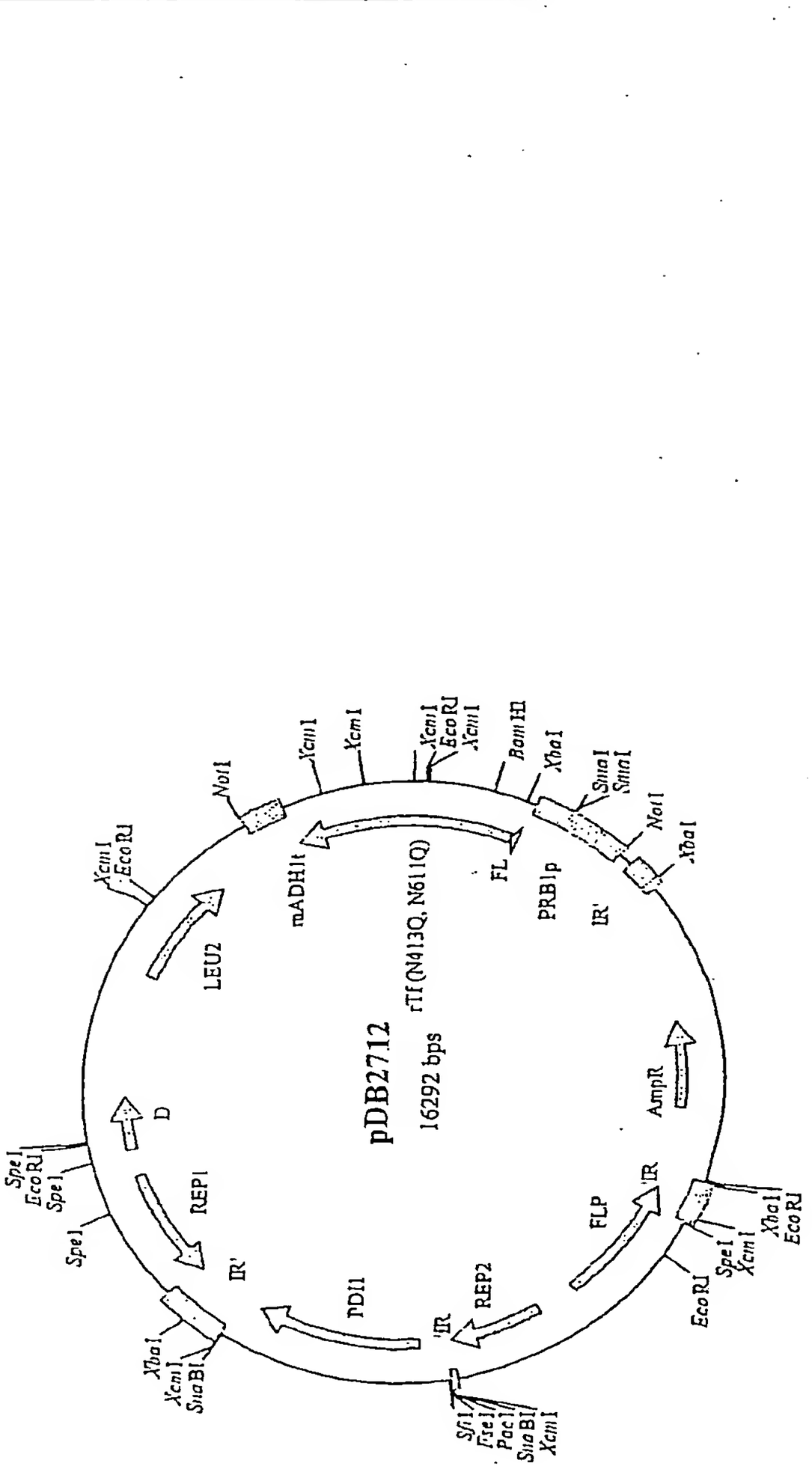


Figure 22

Figure 25

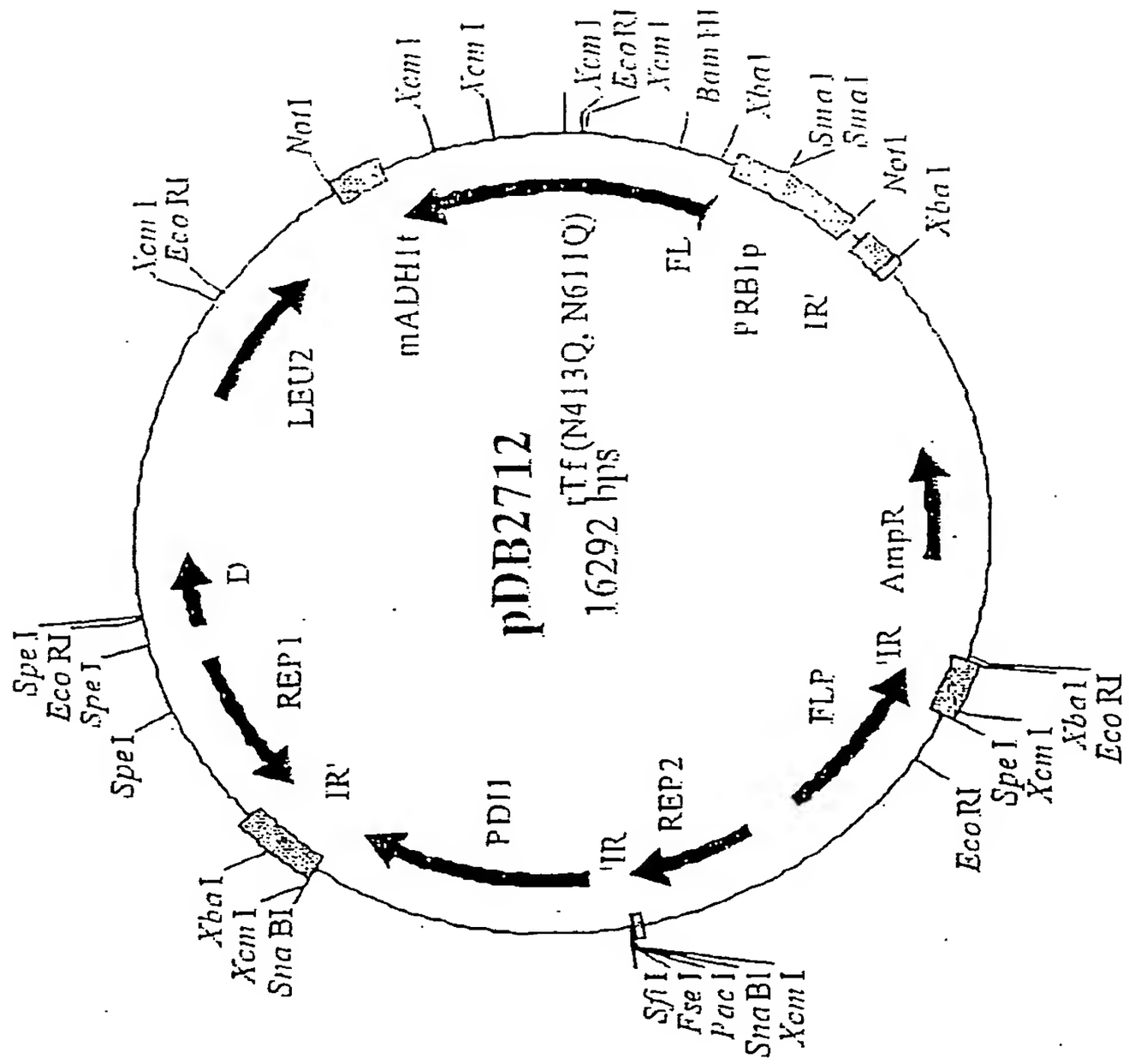


Figure 24

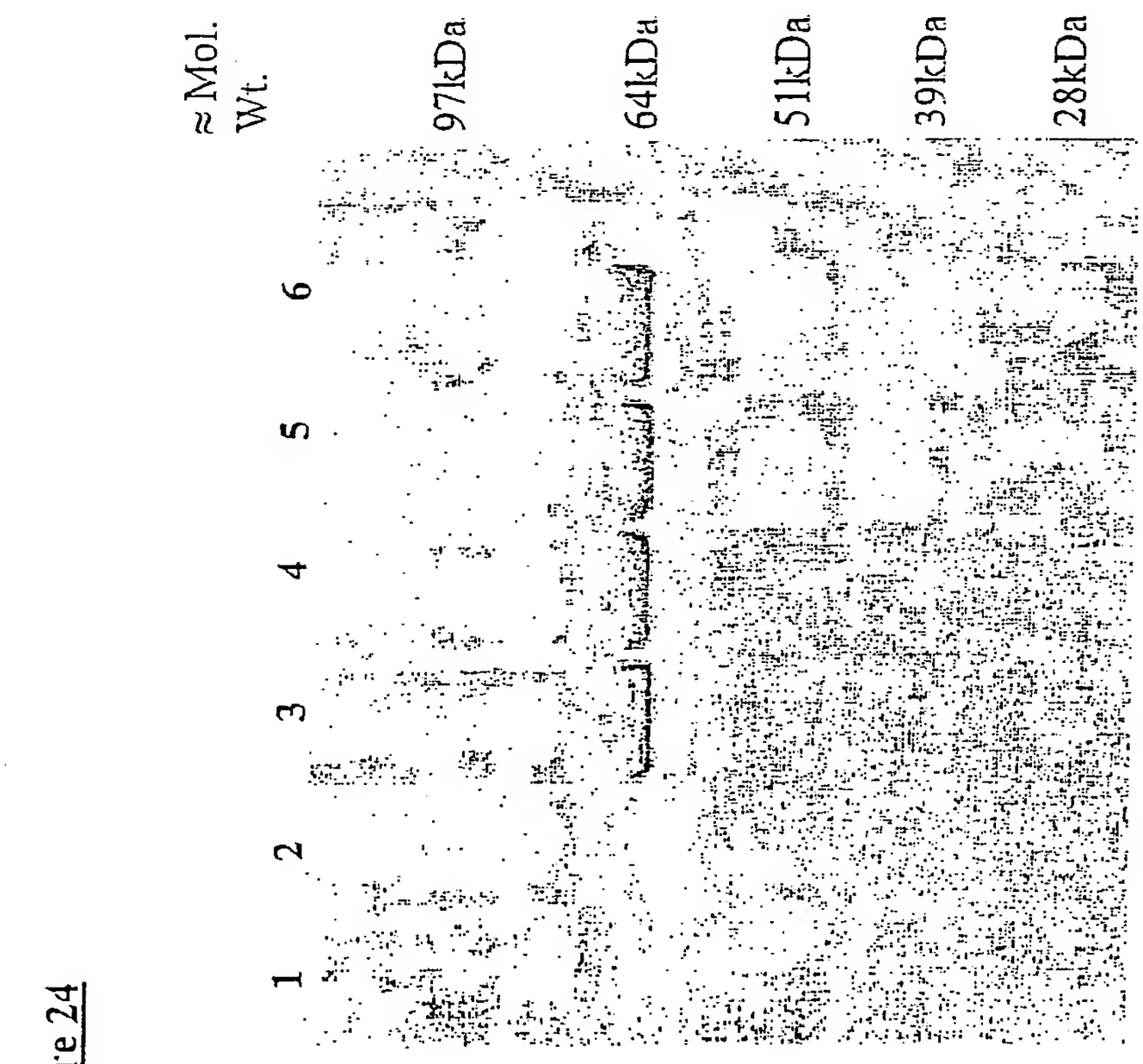
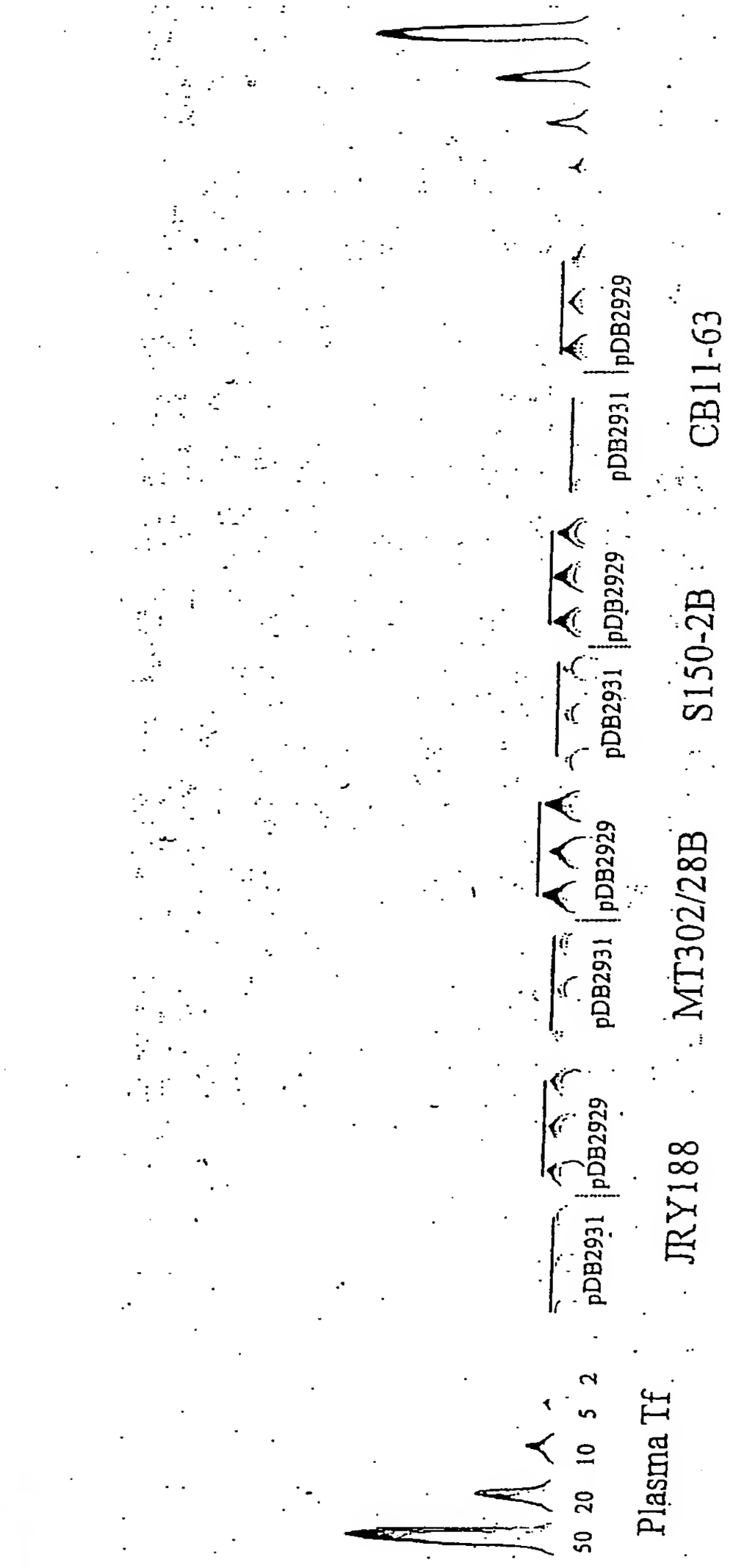


Figure 26


50 20 10 5 2
pDB2931 pDB2929
Plasma Tf
JRY188
MT30
pDB2929 pDB2931 pDB2929 pDB2931 pDB2929 pDB2931
2/28B
S150-2B
CB11-63

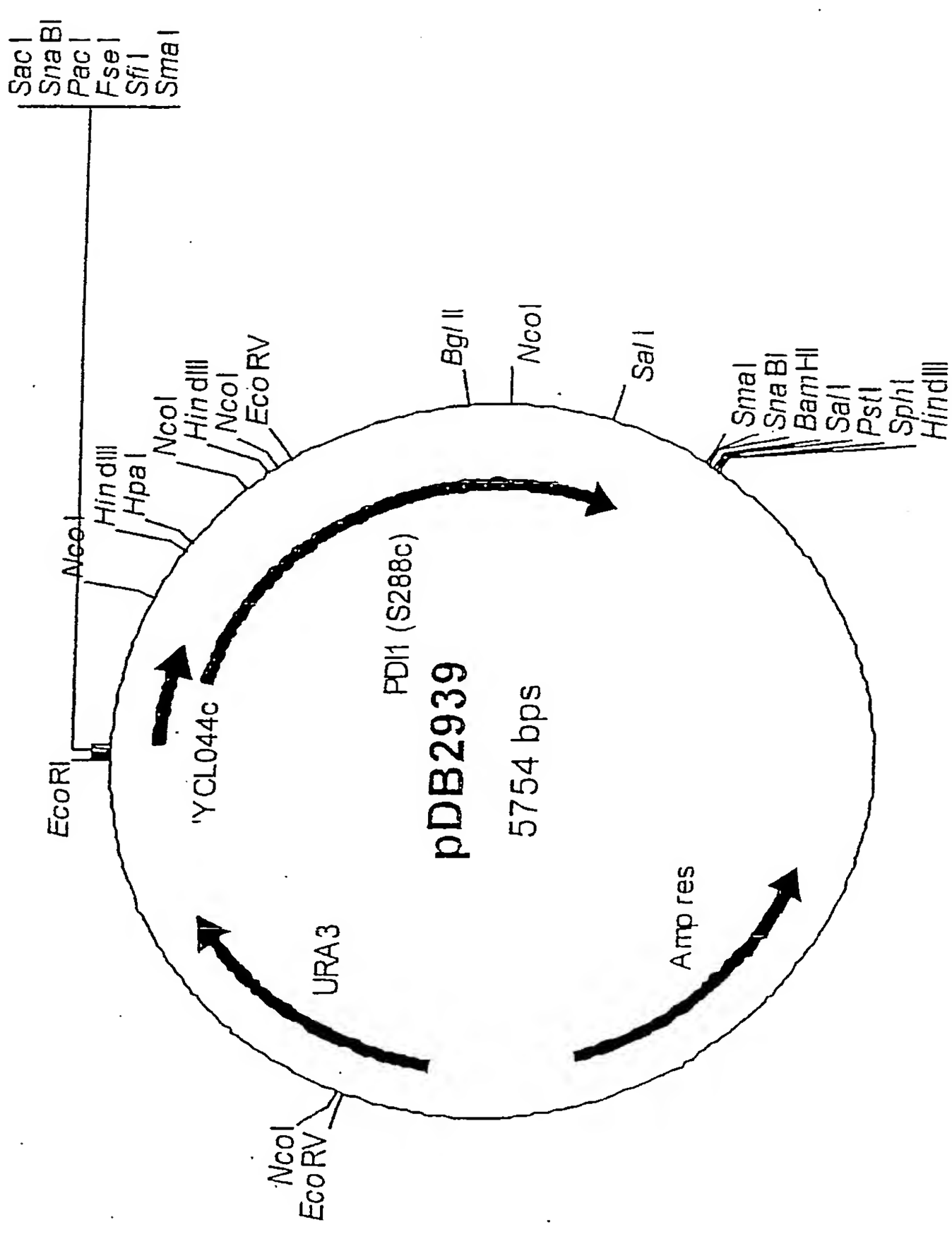

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
Not I
Hin dIII
Hpa I
Nco I
Hin dIII
Nco I
Eco RV
Bgl II
Nco I
Sal I
Sma I
Sna BI
Bam HI
Sal I
Pst I
Sph I
Hin dIII
EcoRI
YCL044c
PDI1 (S288c)
pDB2939
5754 bps
URA3
Amp res
Nco I
Eco RV

Figure 27

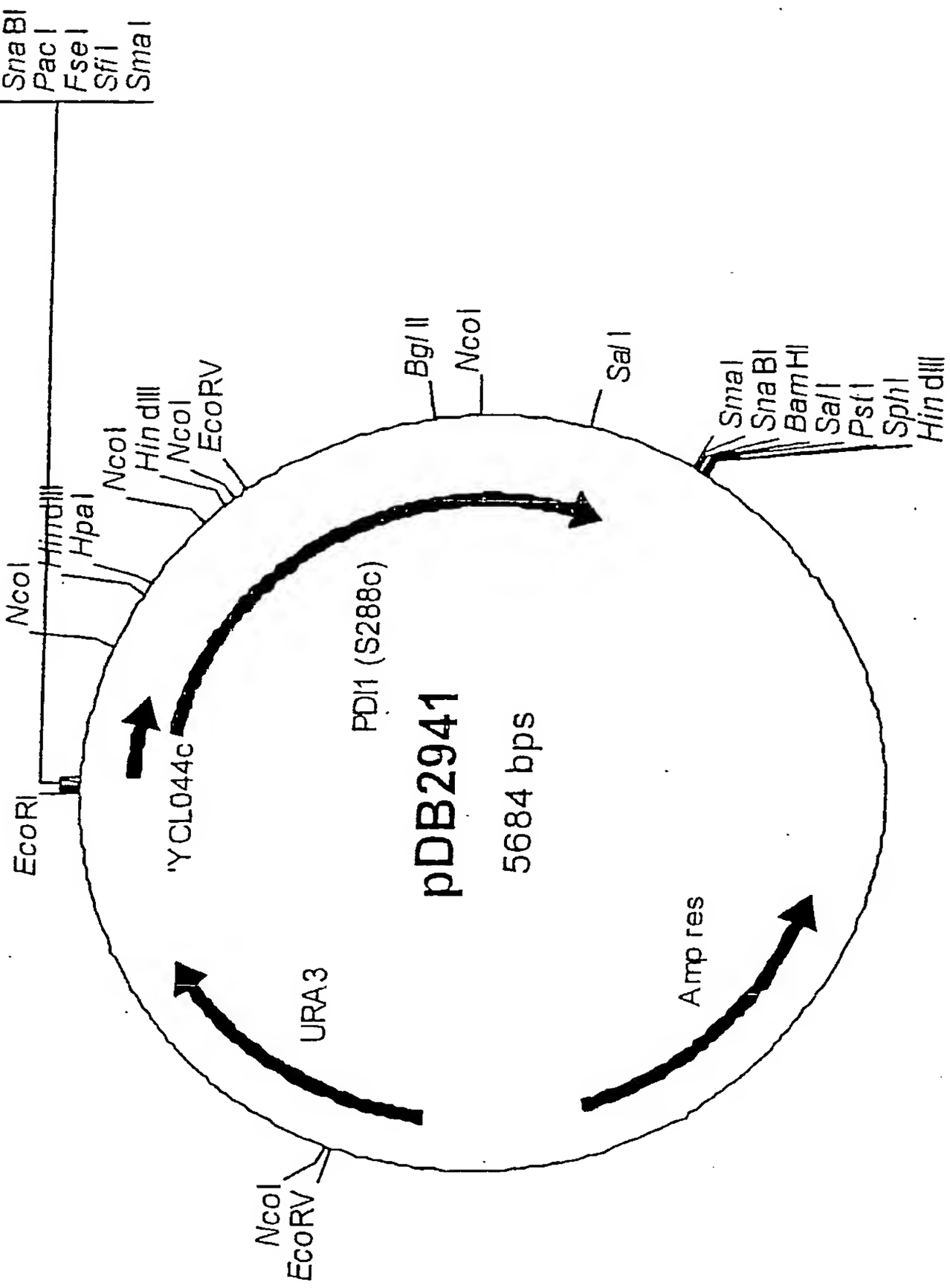

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
Bgl II
Nco I
Sal I
Sma I
Sna BI
Bam HI
Sal I
Pst I
Sph I
Hin dIII
Nco I
Hin dIII
Hpa I
Nco I
Hin dIII
Nco I
EcoRV
EcoRI
'YCL044c
PDI1 (S288c)
PDI1
pDB2941
5684 bps
URA3
Amp res
Nco I
EcoRV

Figure 28

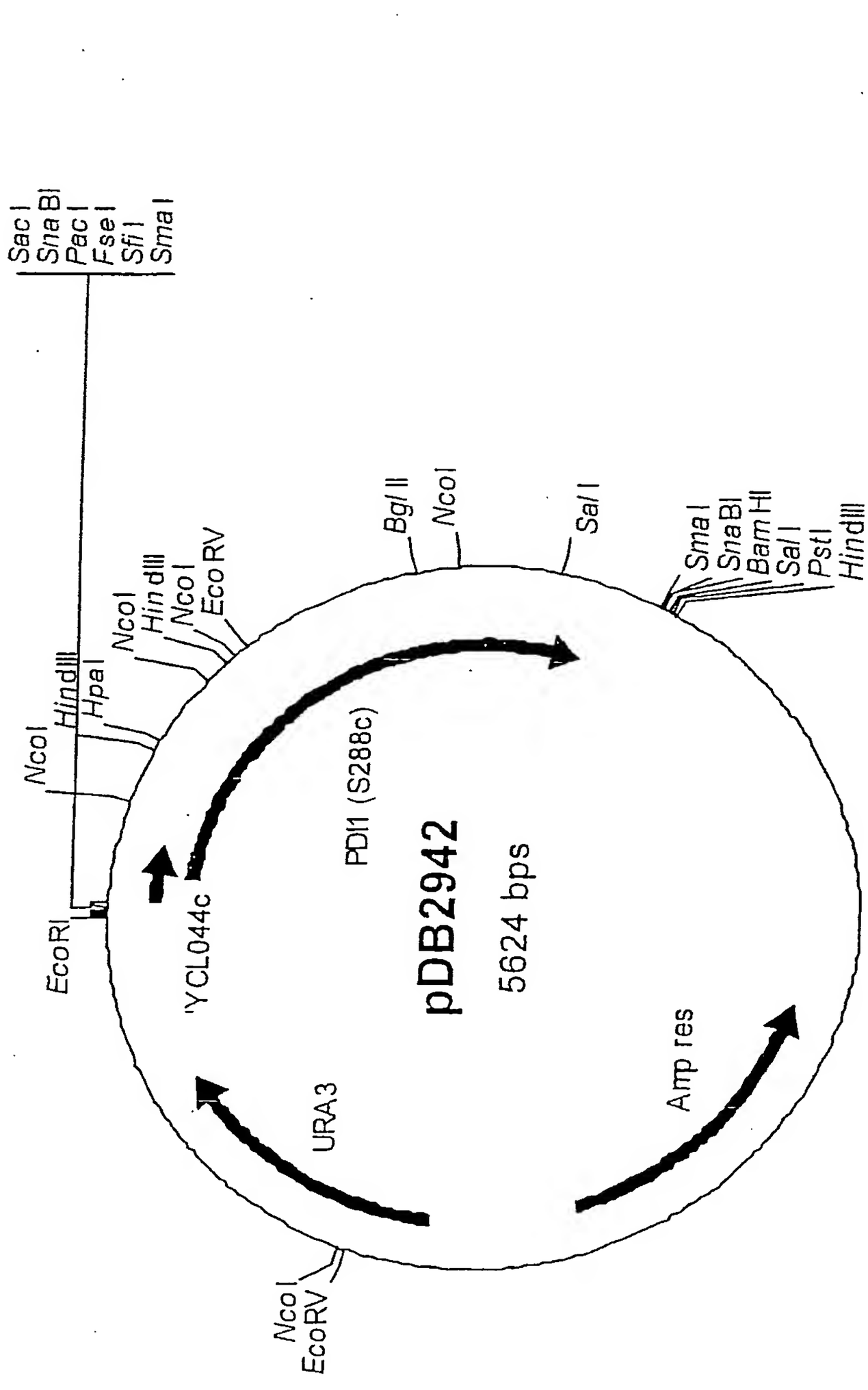

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
Hind III
Hpa I
Nco I
Nco I
Hin dIII
Nco I
Eco RV
Bgl II
Nco I
Sal I
Sma I
Sna BI
Bam HI
Sal I
Pst I
Hind III
EcoRI
YCL044c
PDI1 (S288c)
pDB2942
5624 bps
URA3
Amp res
Nco I
EcoRV

Figure 29

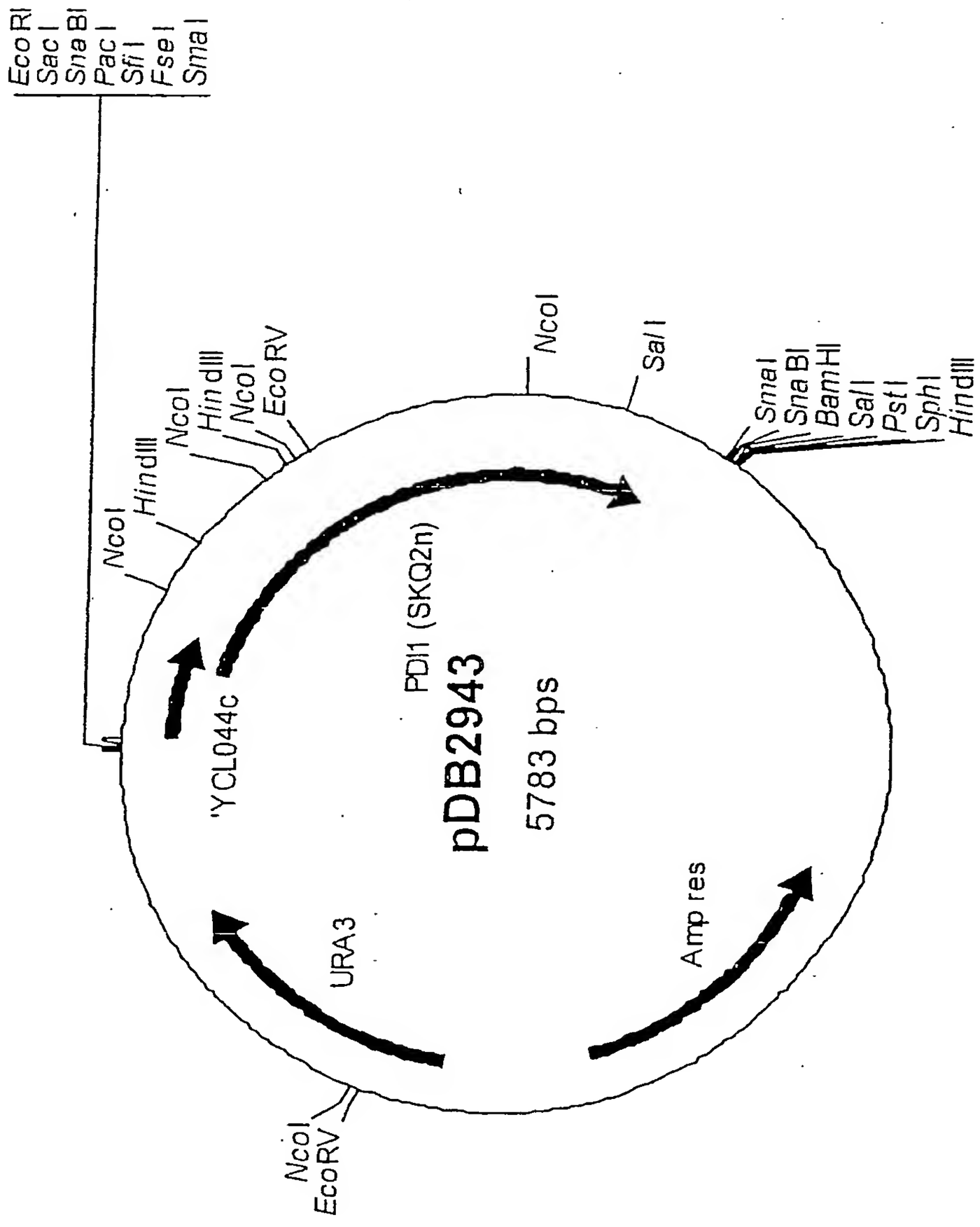

EcoRI
SacI
SnaBI
PacI
SfiI
FseI
SmaI
NcoI
HindIII
NcoI
HindIII
NcoI
EcoRV
NcoI
SalI
SmaI
SnaBI
BamHI
SalI
PstI
SphI
HindIII
YCL044c
PDI1 (SKQ2n)
pDB2943
5783 bps
URA3
Amp res
NcoI
EcoRV

Figure 30

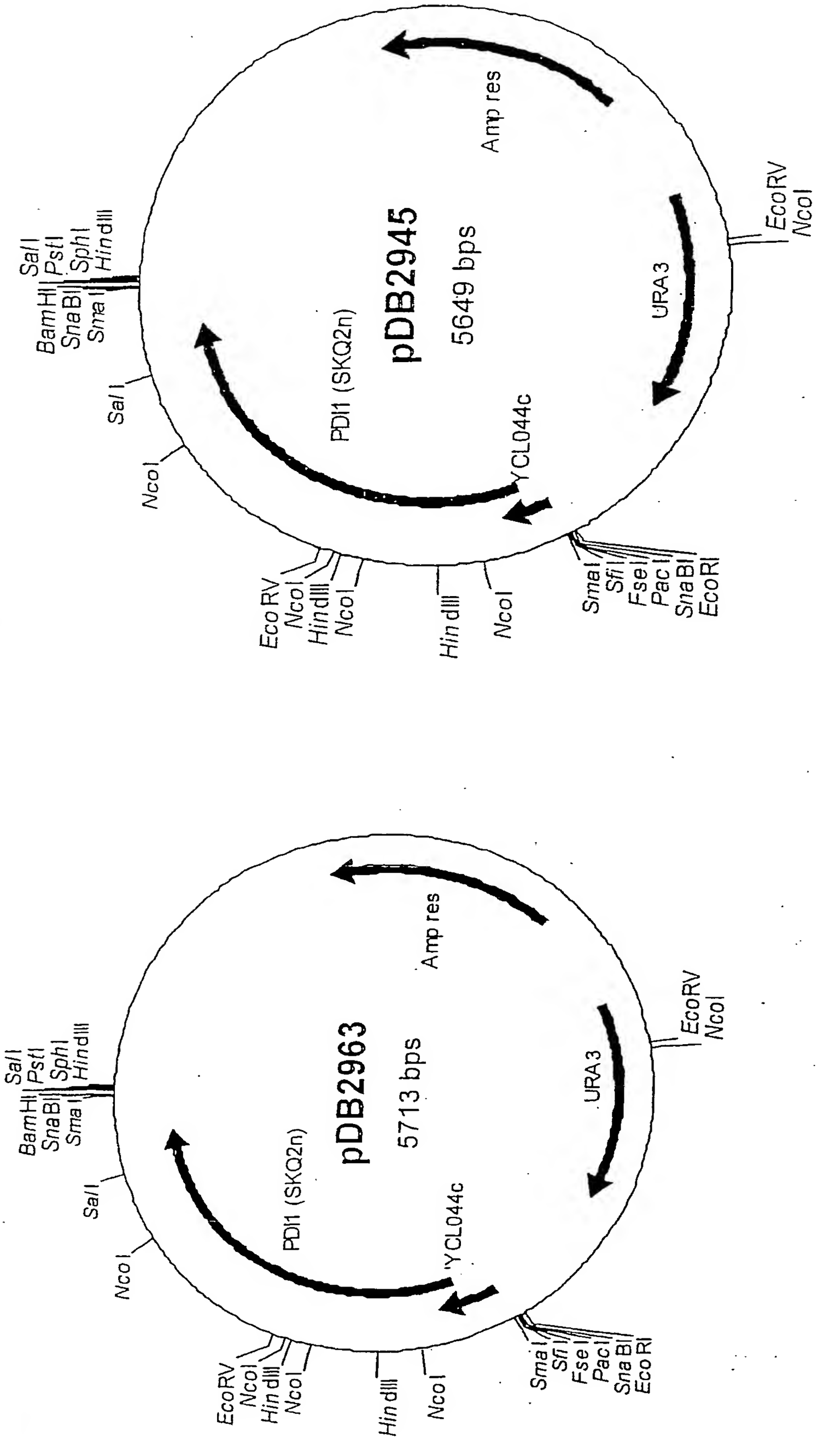


Figure 32

Figure 31

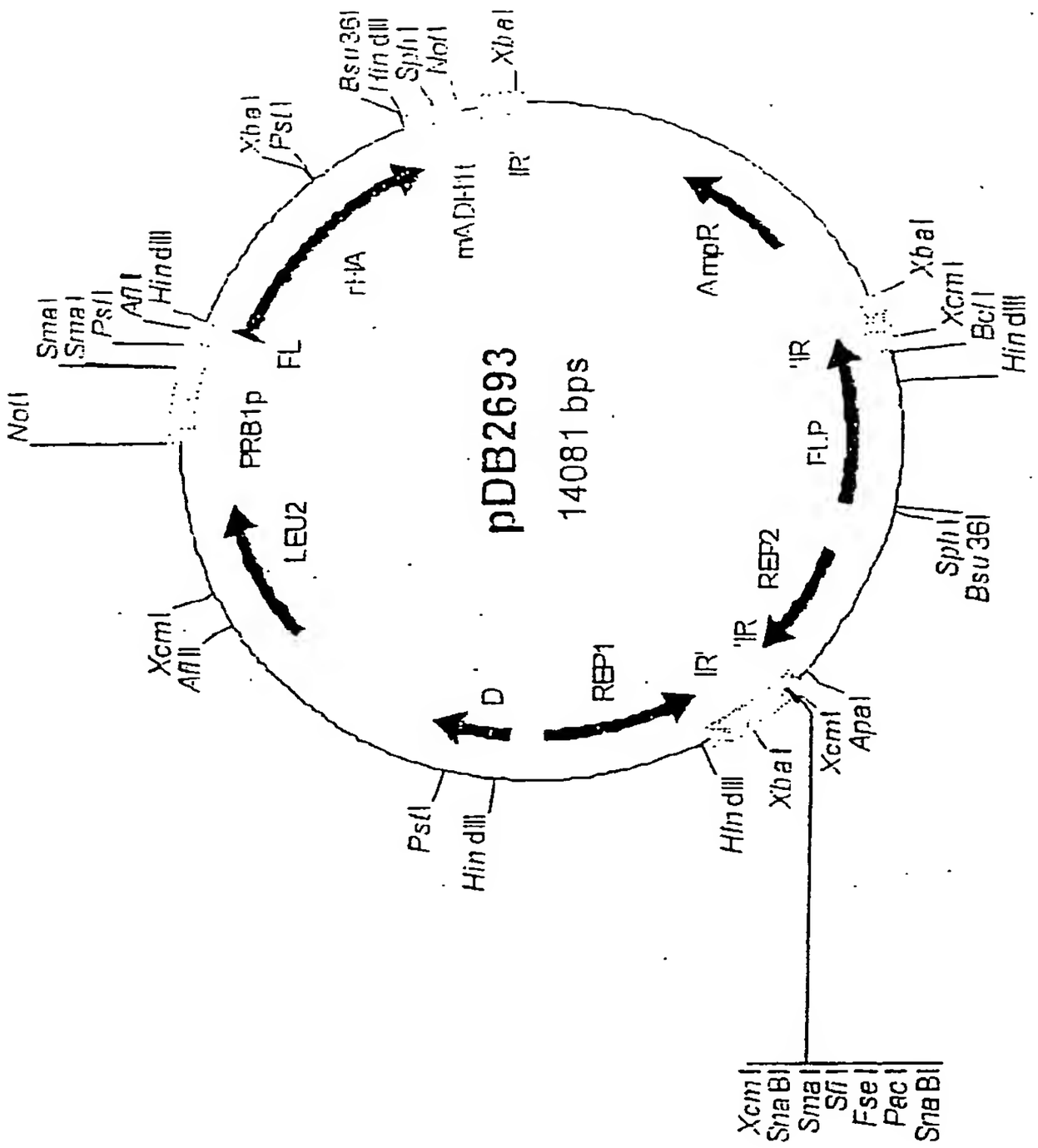


Figure 34

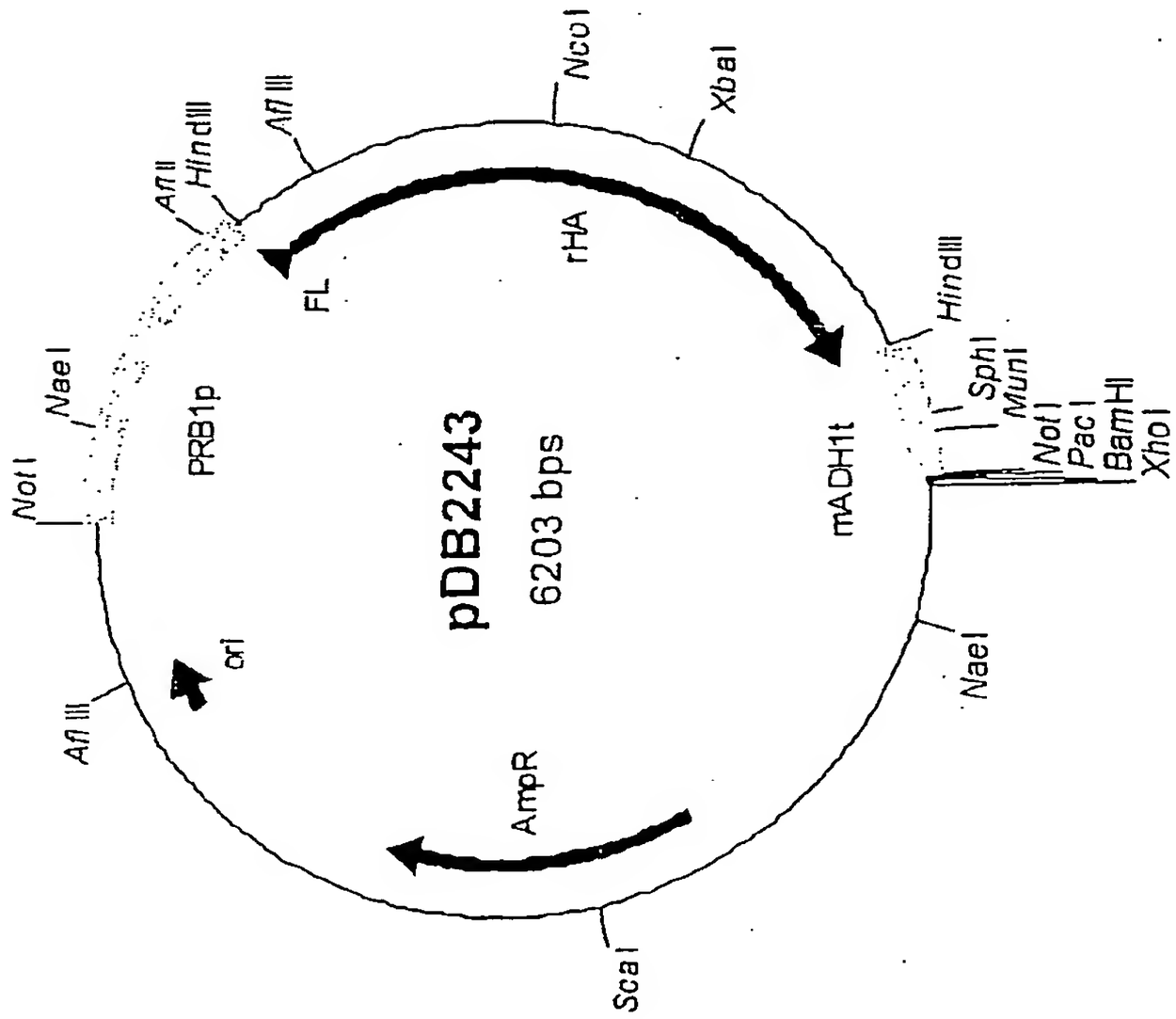


Figure 33

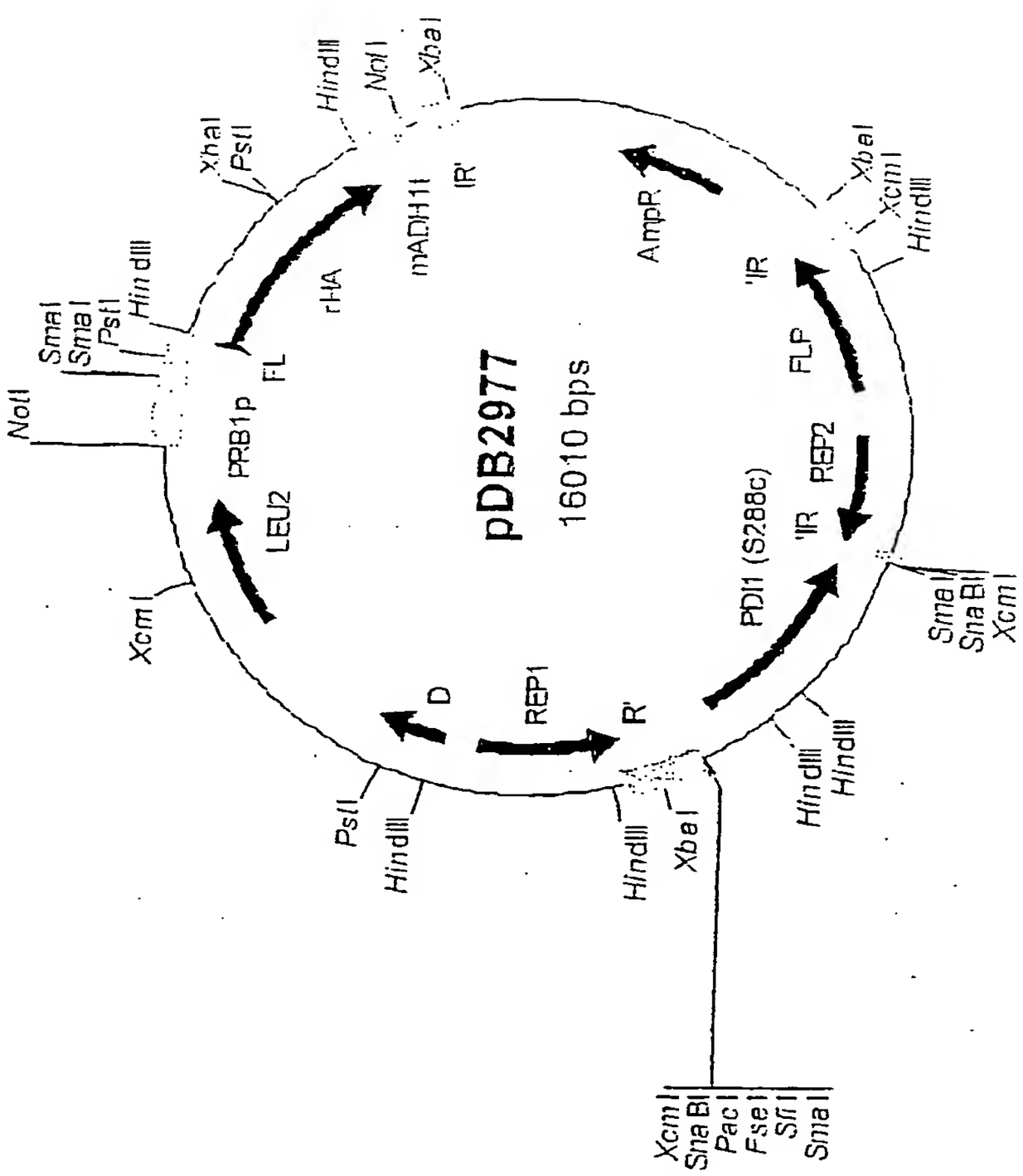


FIGURE 36

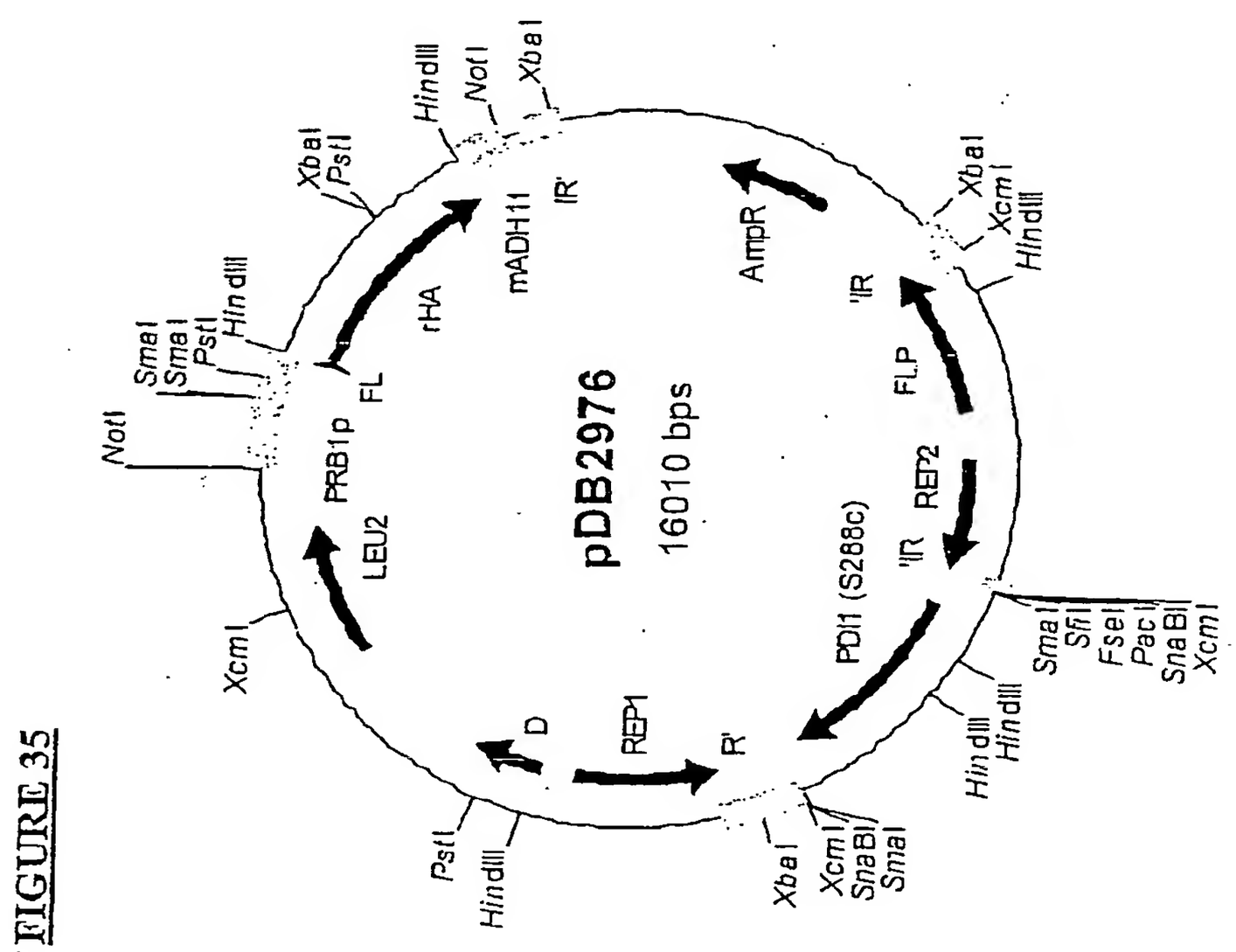


FIGURE 35

FIGURE 38

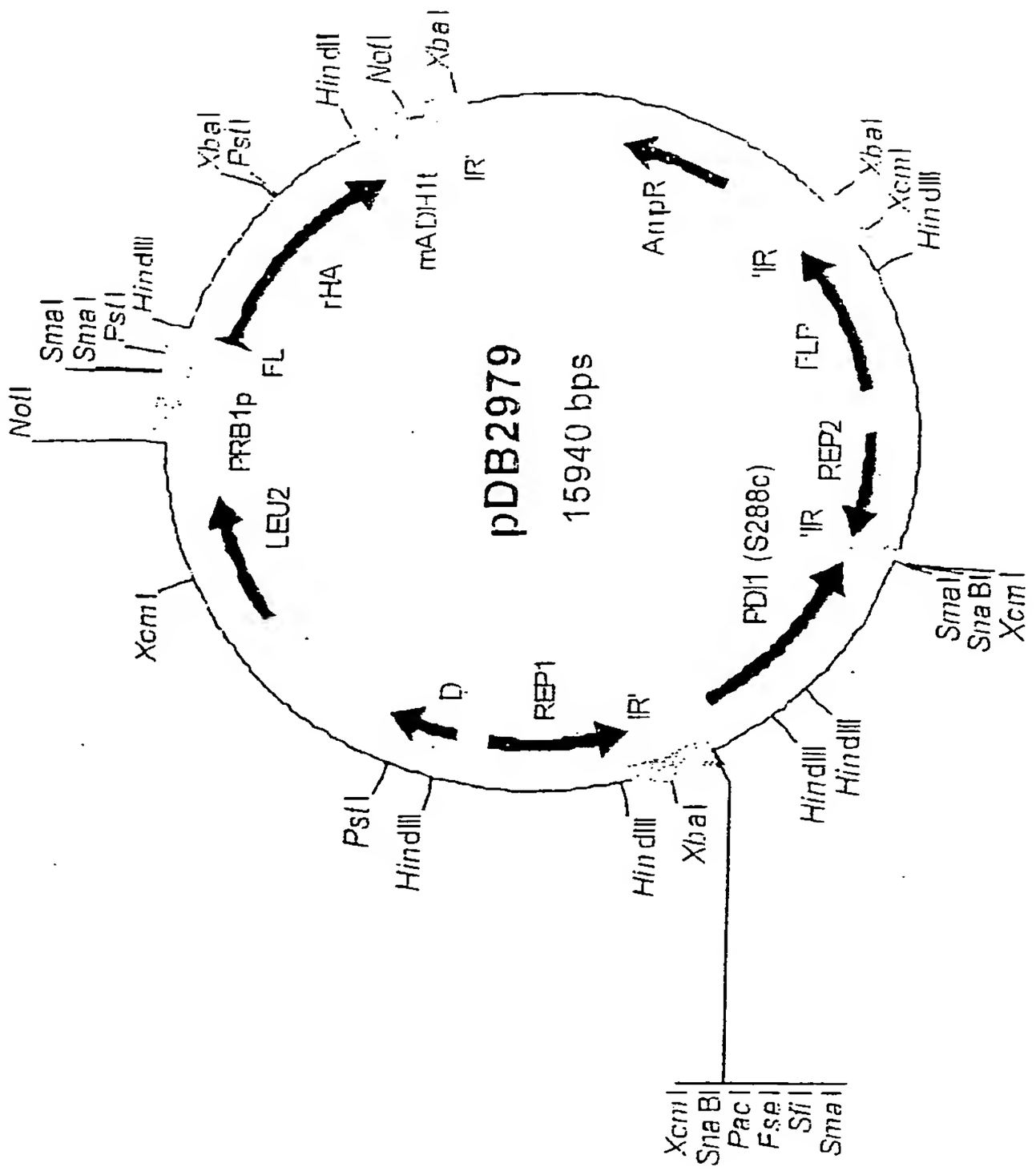
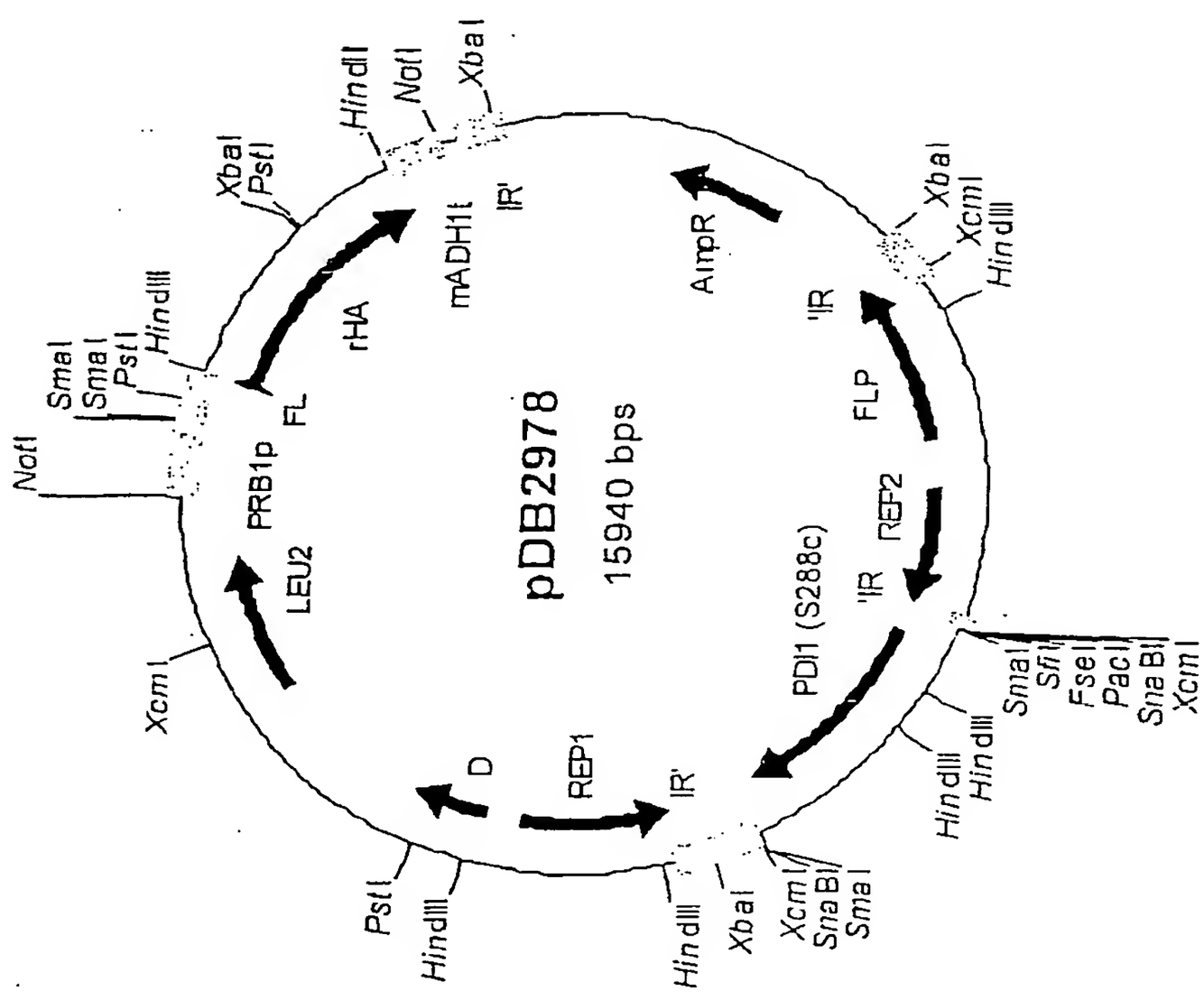


FIGURE 37



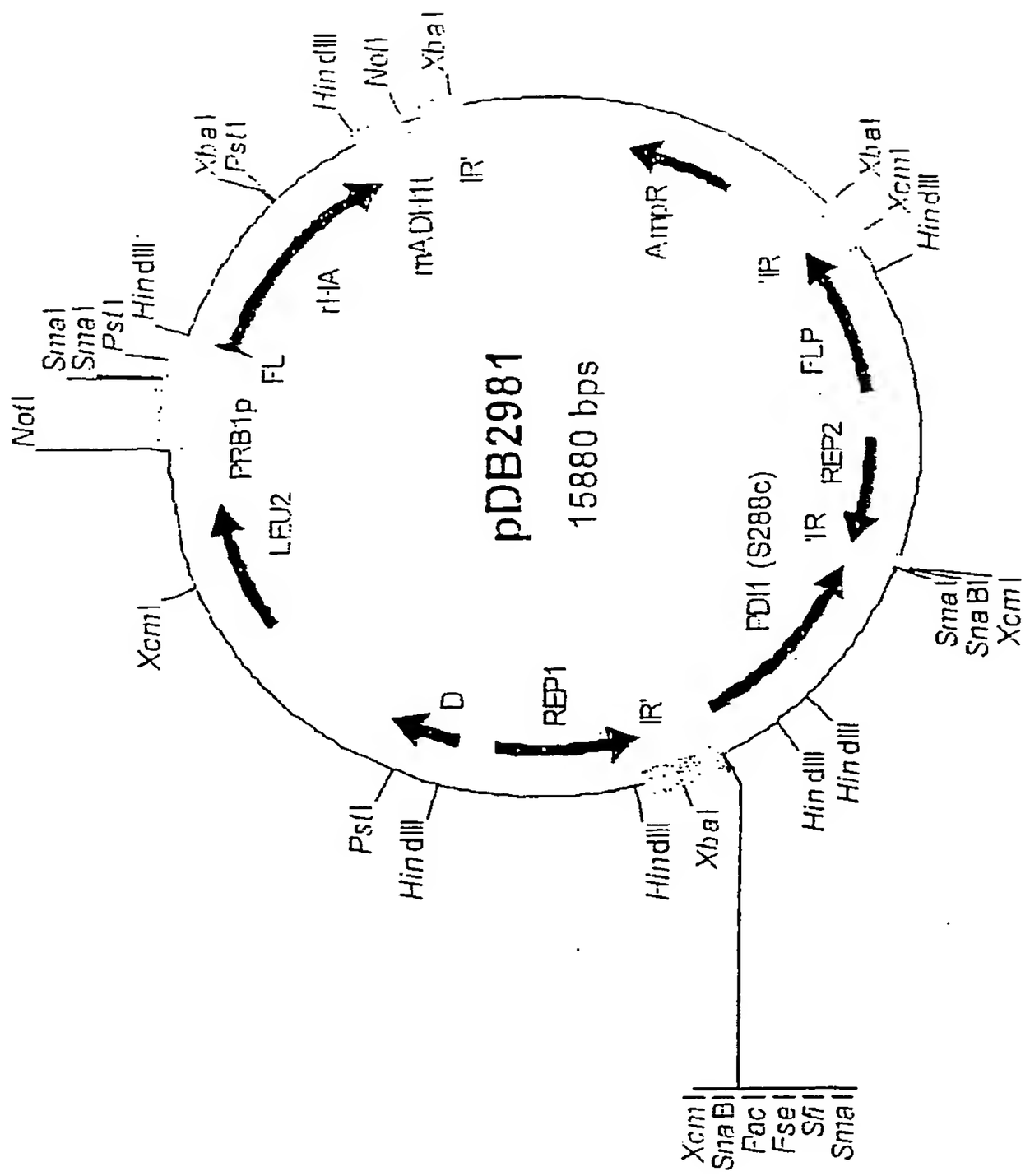


FIGURE 40

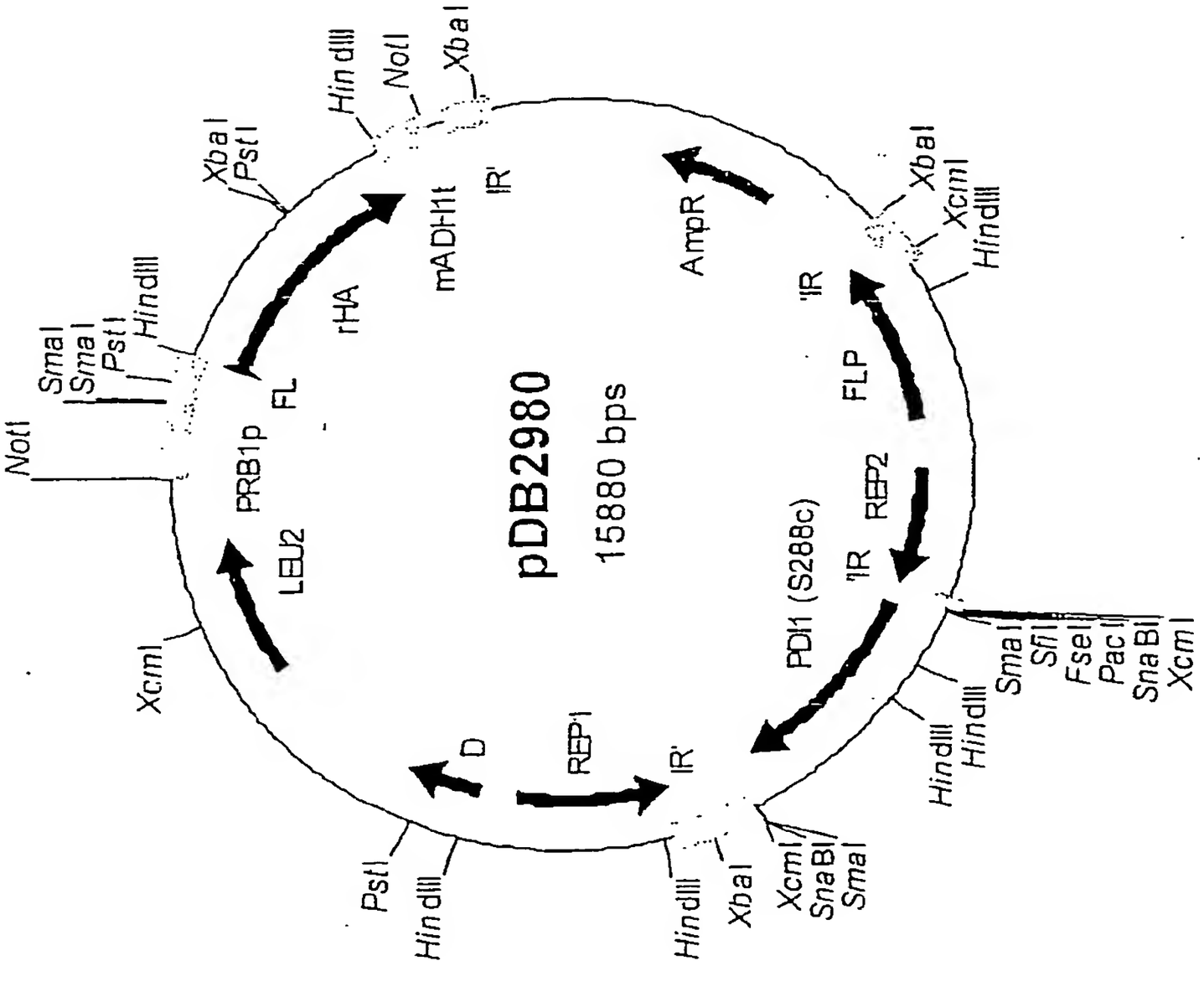


FIGURE 39

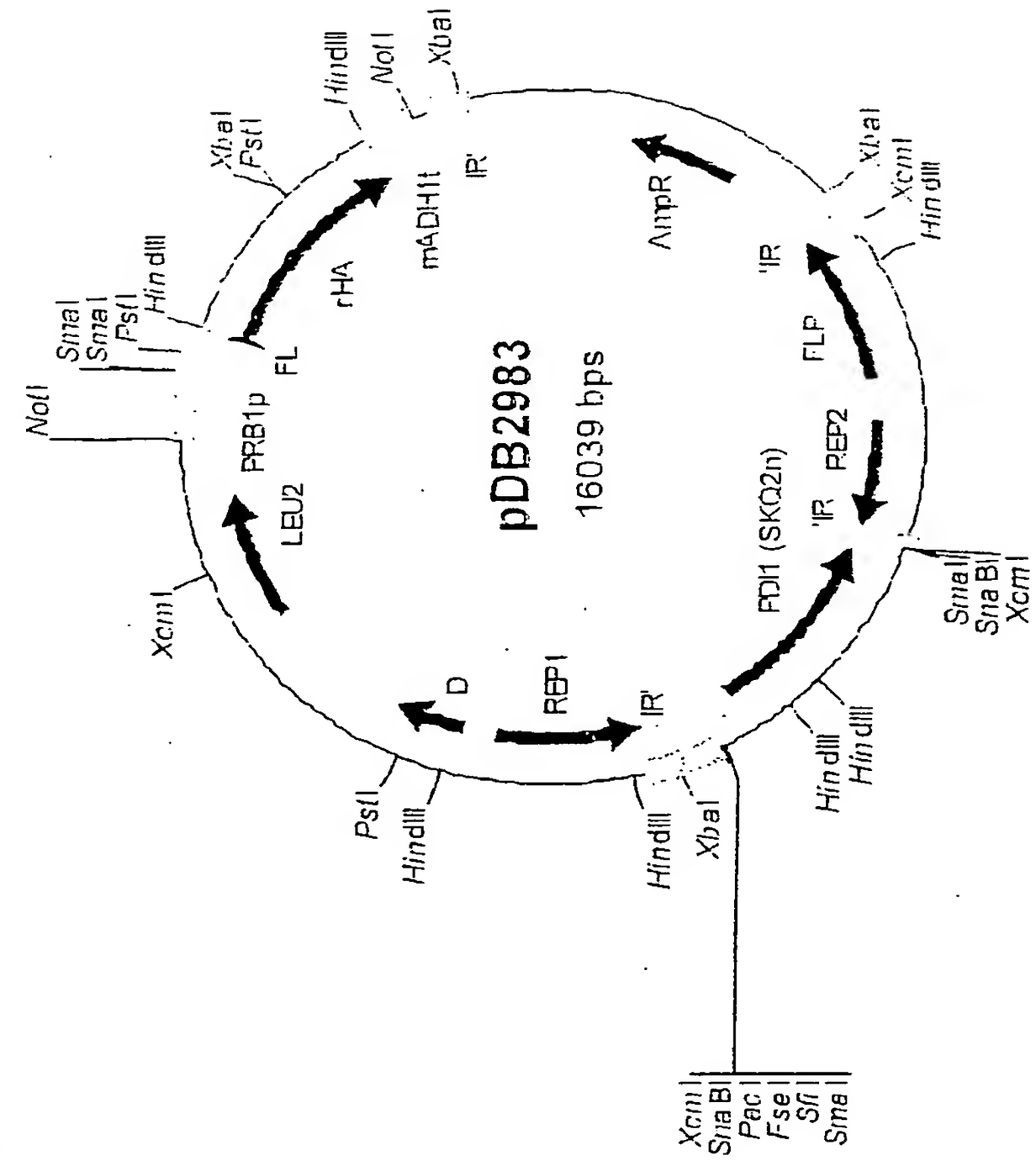


FIGURE 42

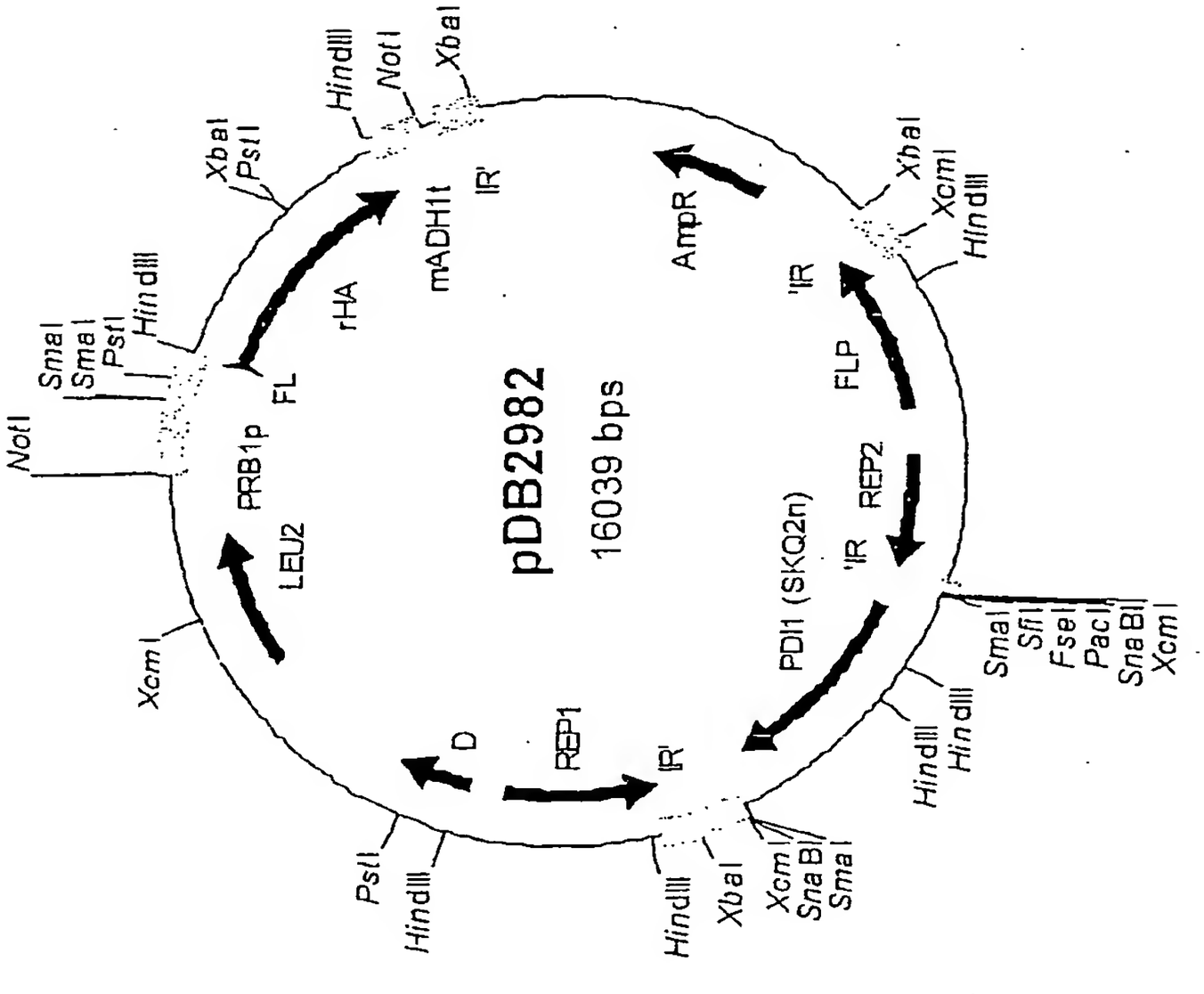


FIGURE 41

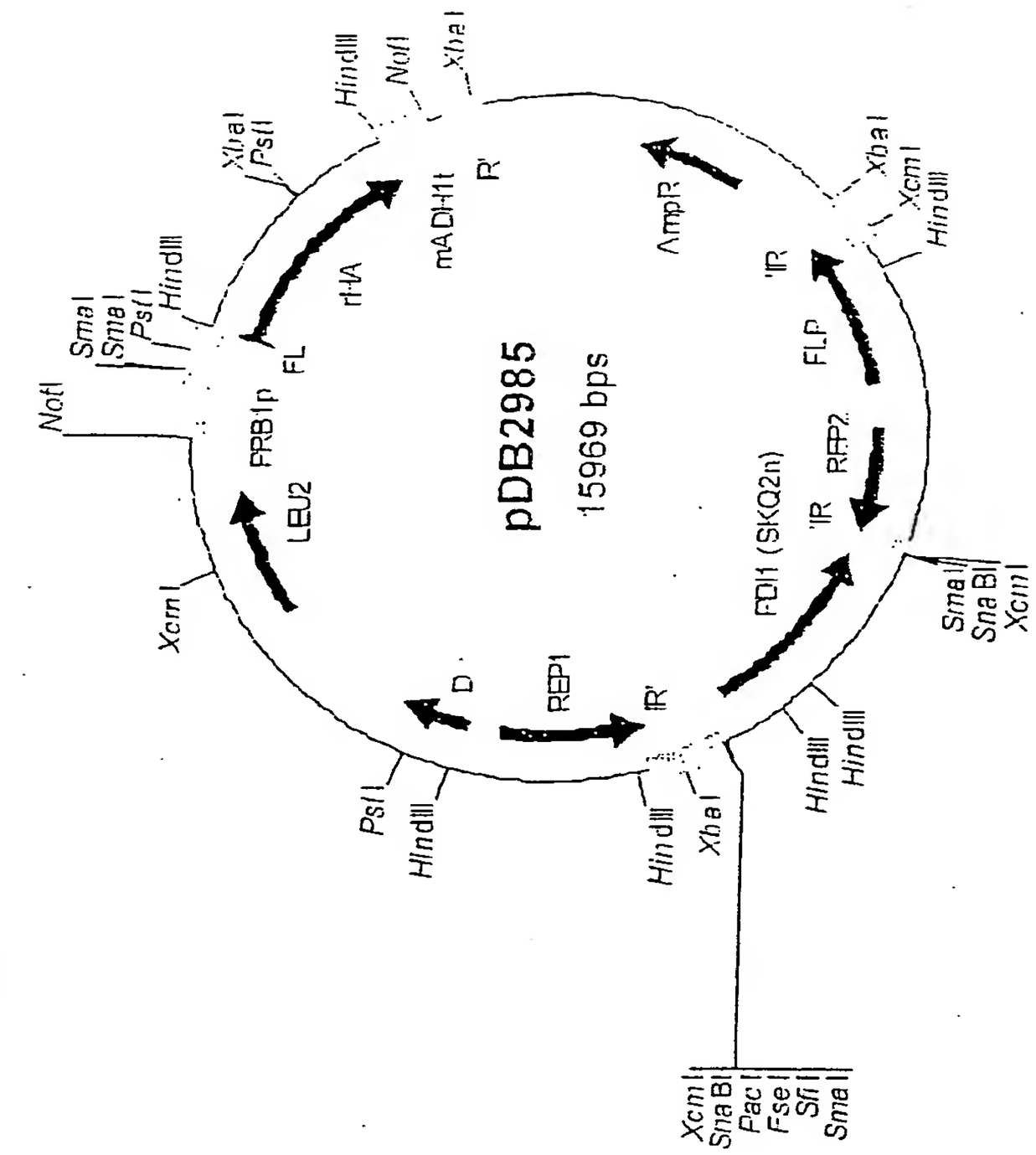


FIGURE 44

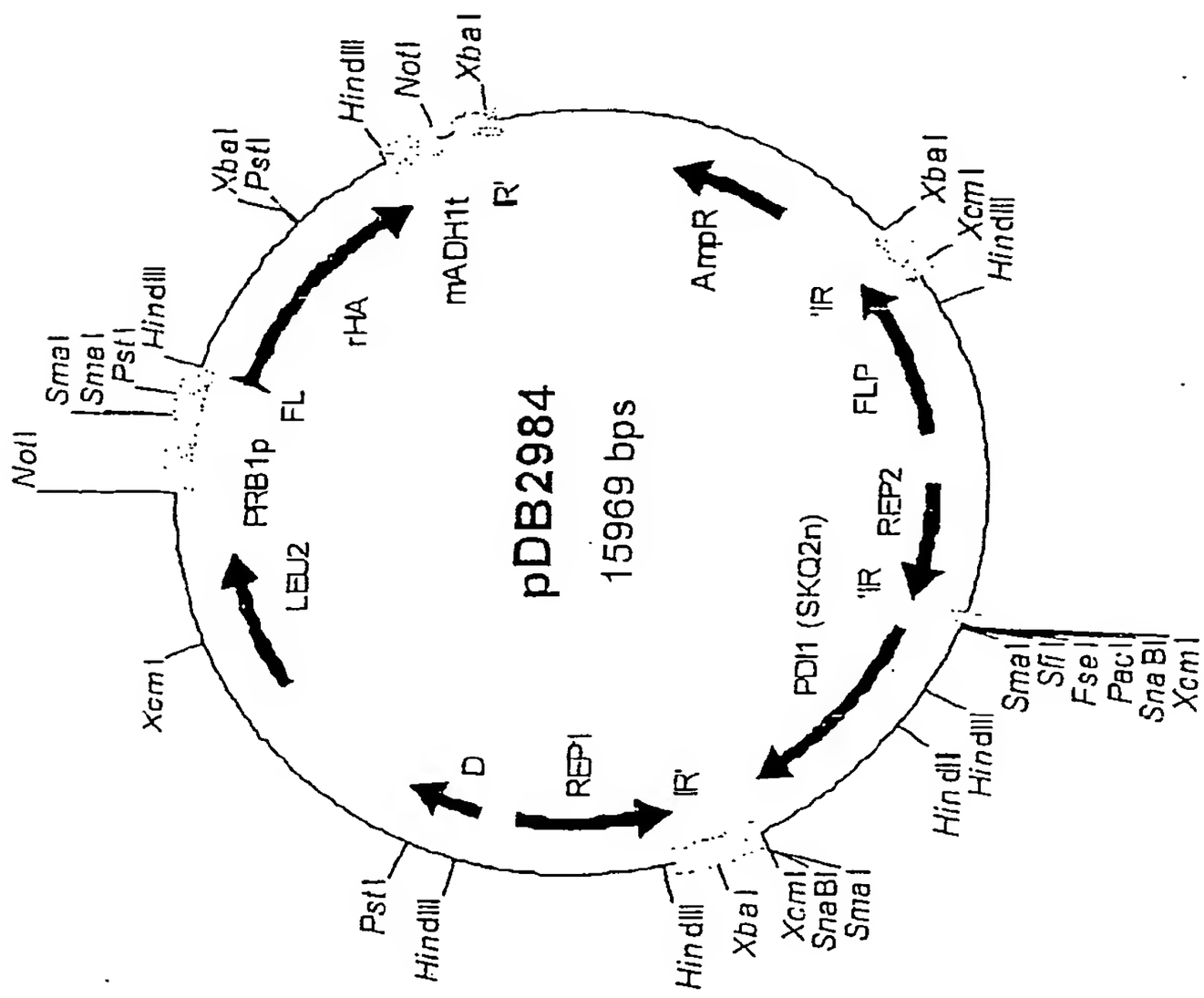


FIGURE 43

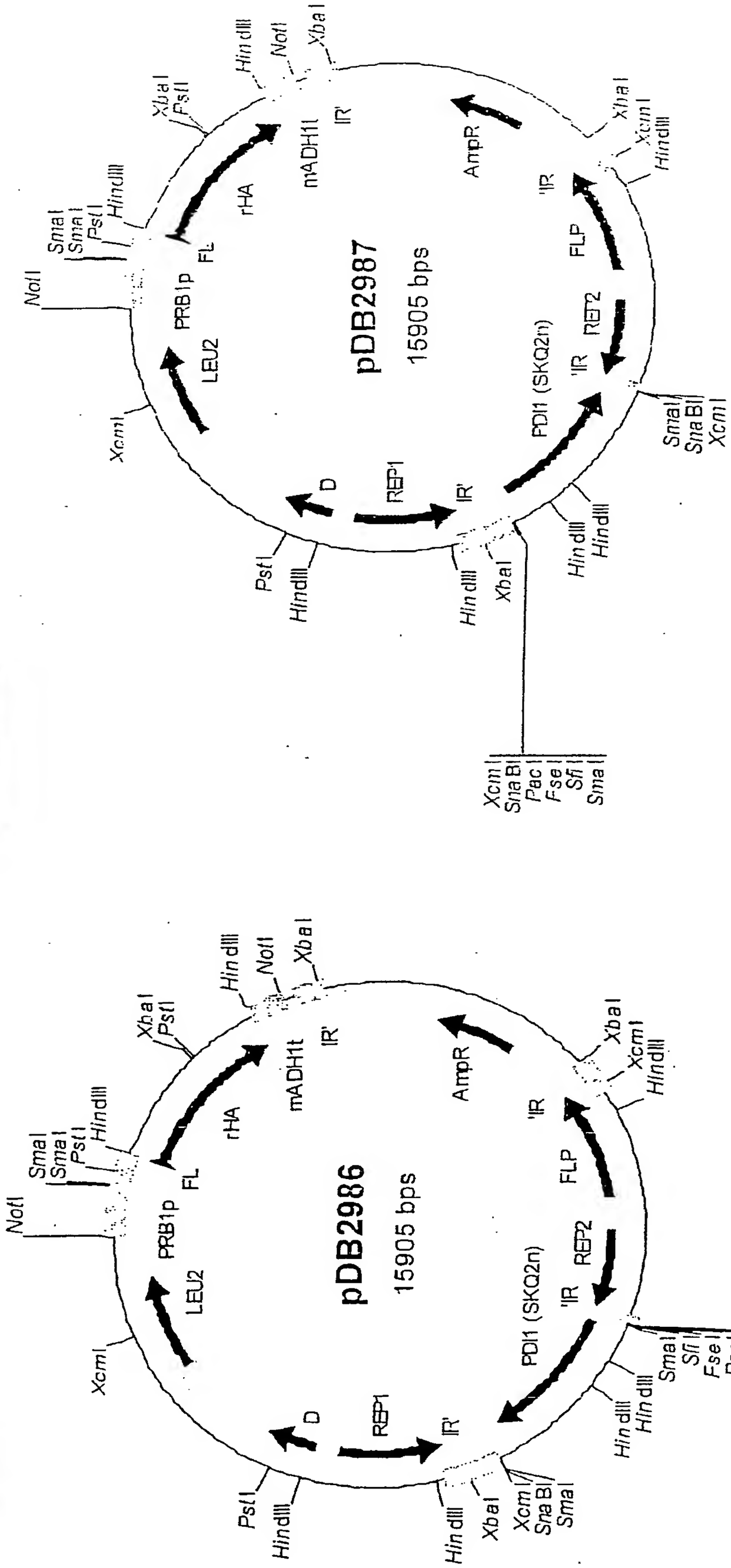


FIGURE 46

FIGURE 45

FIGURE 48

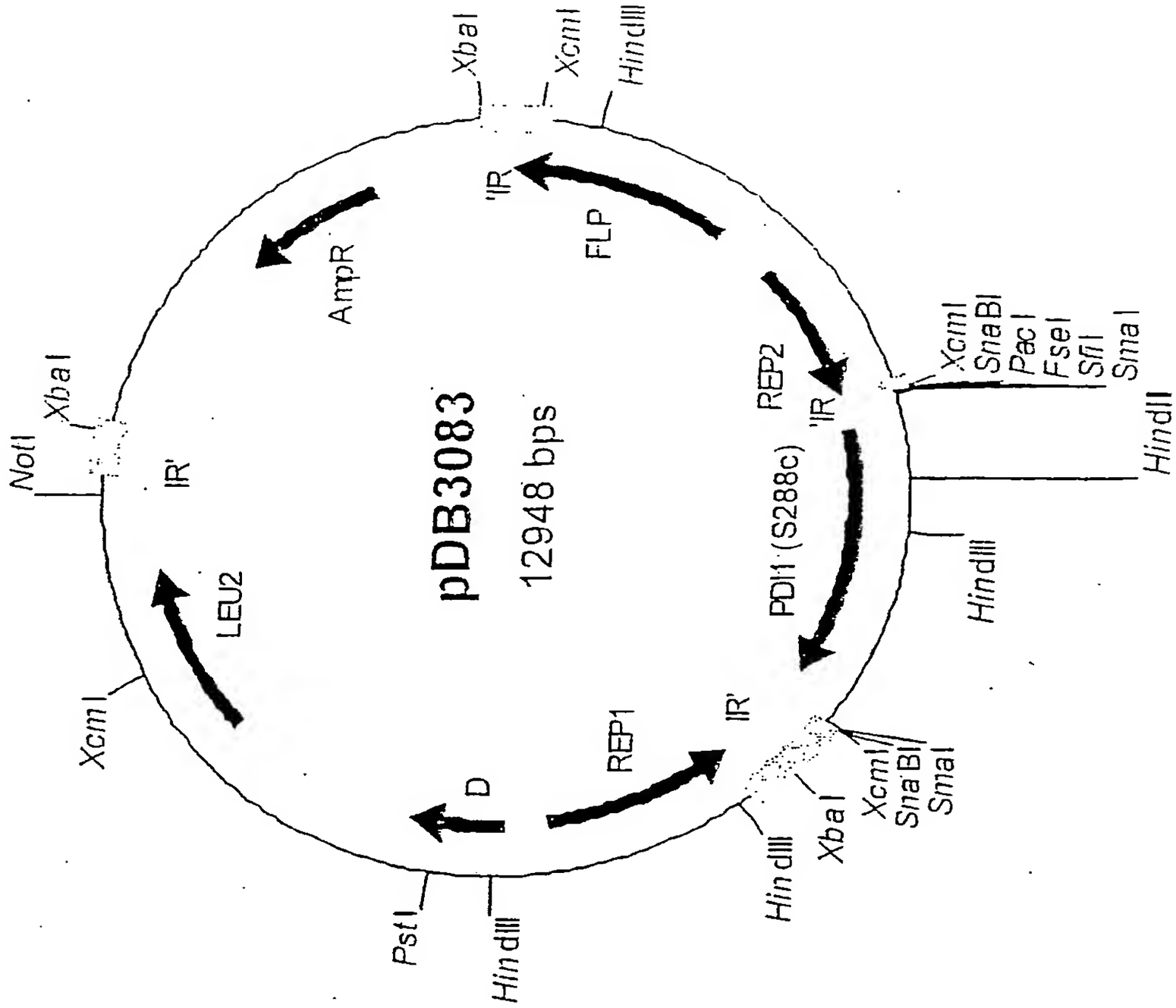
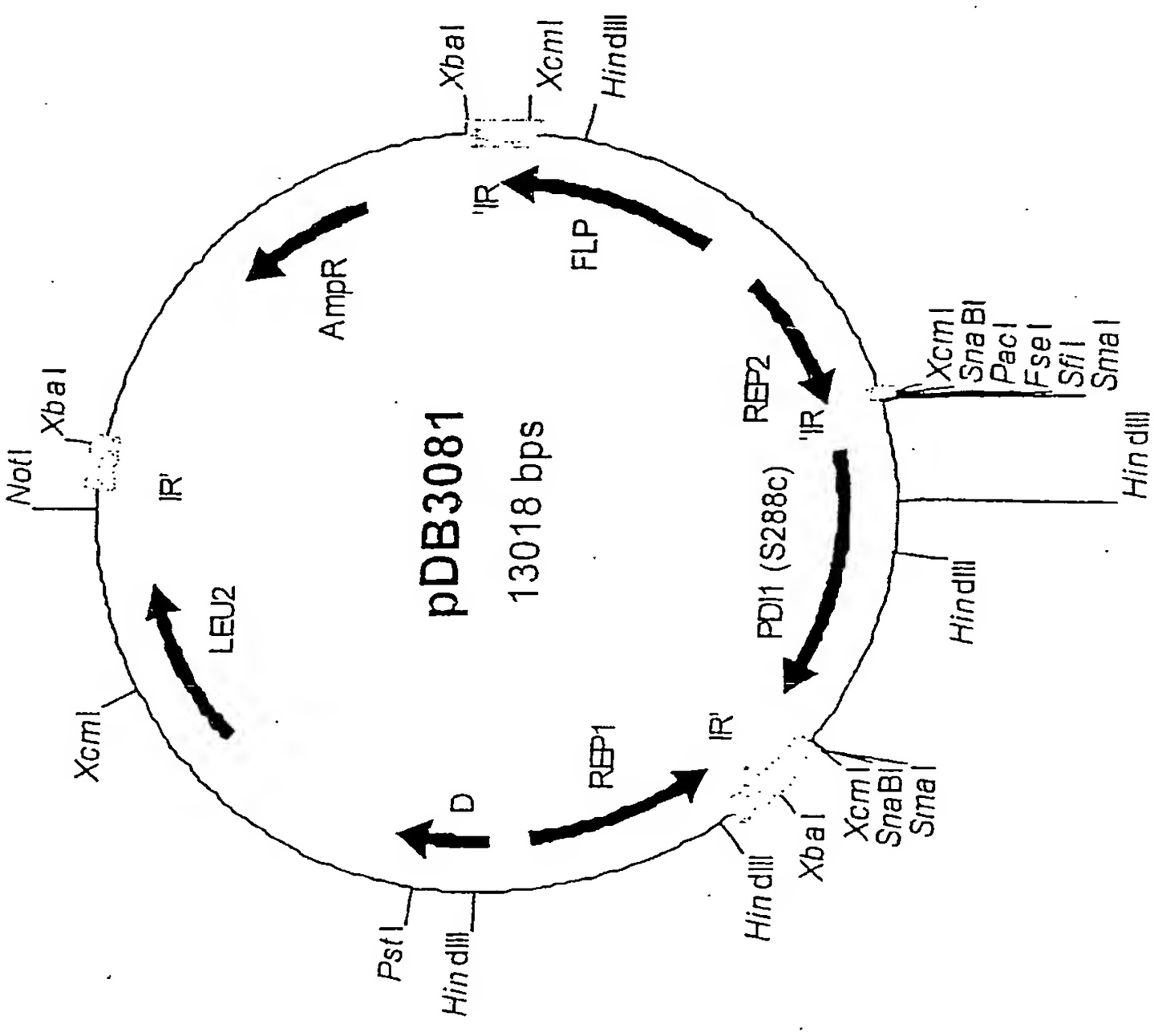


FIGURE 47



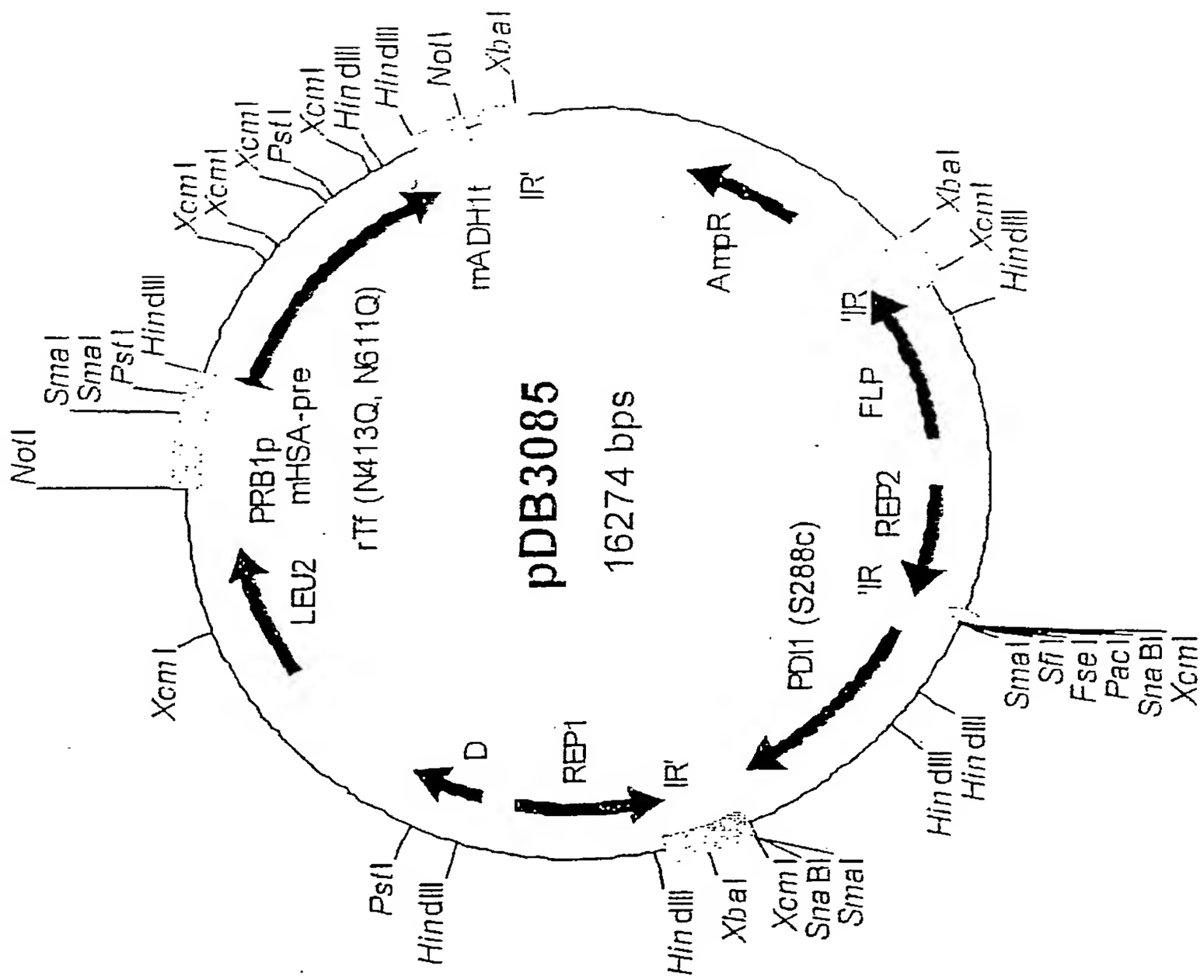


FIGURE 50

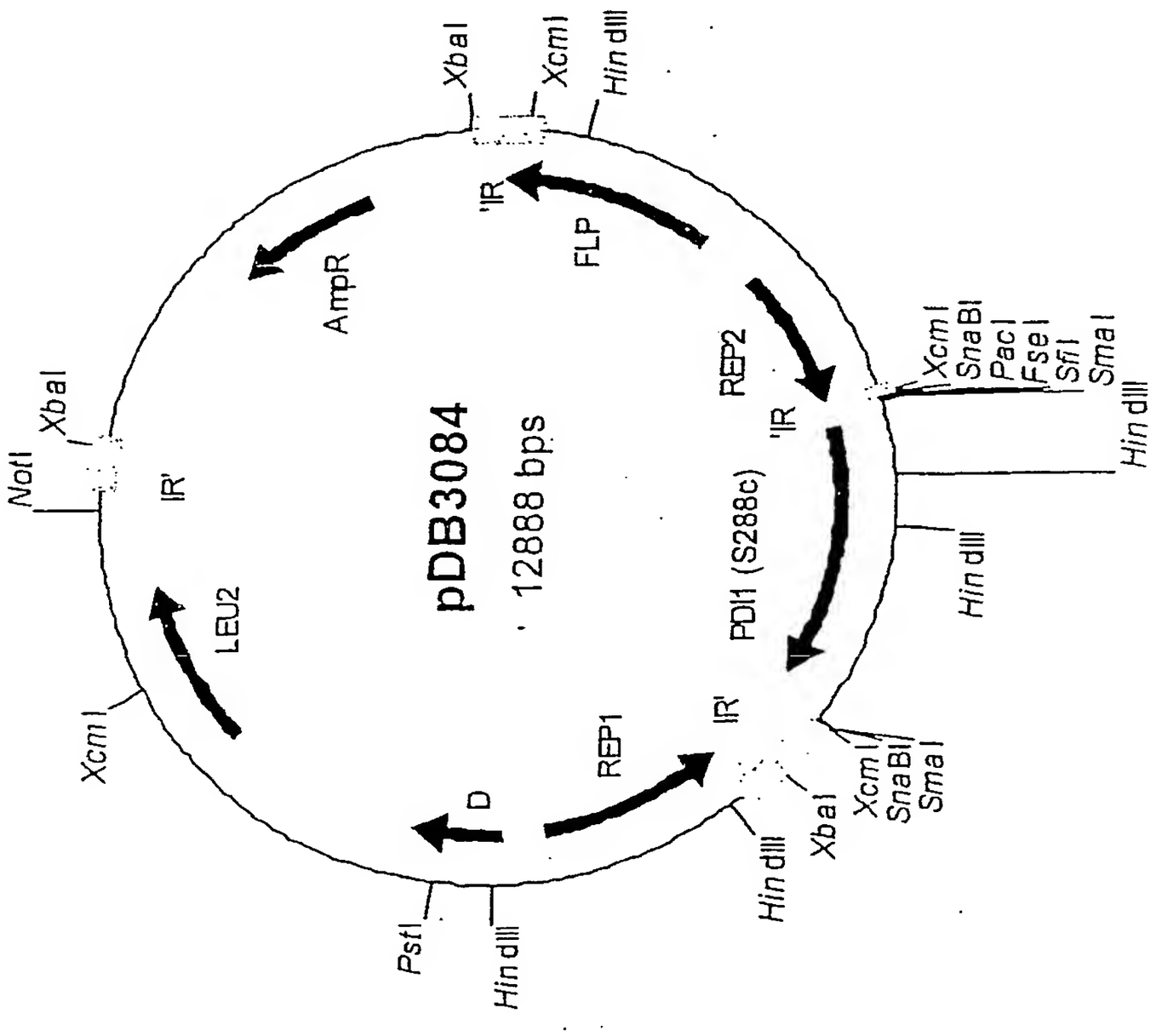


FIGURE 49

FIGURE 51

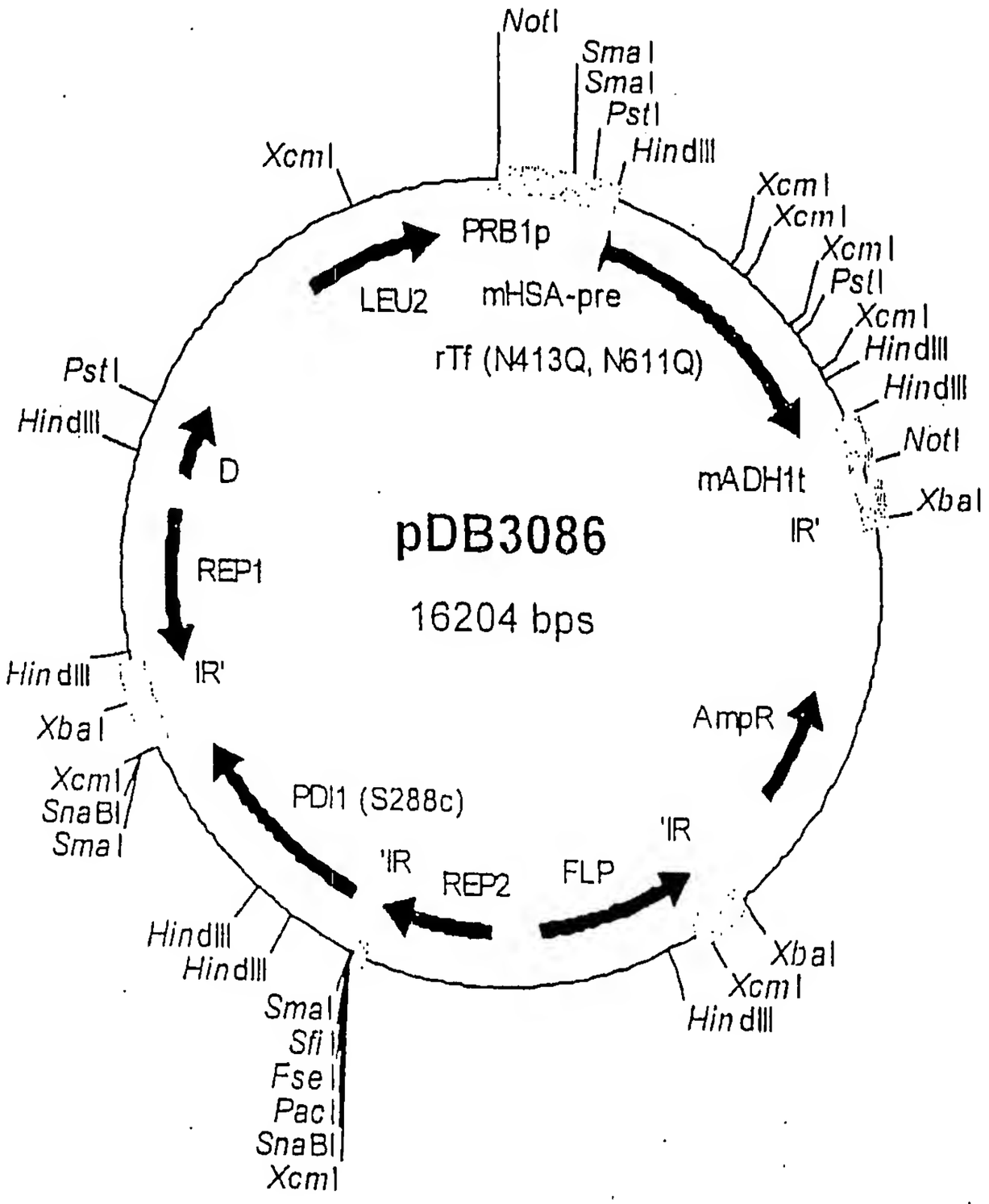
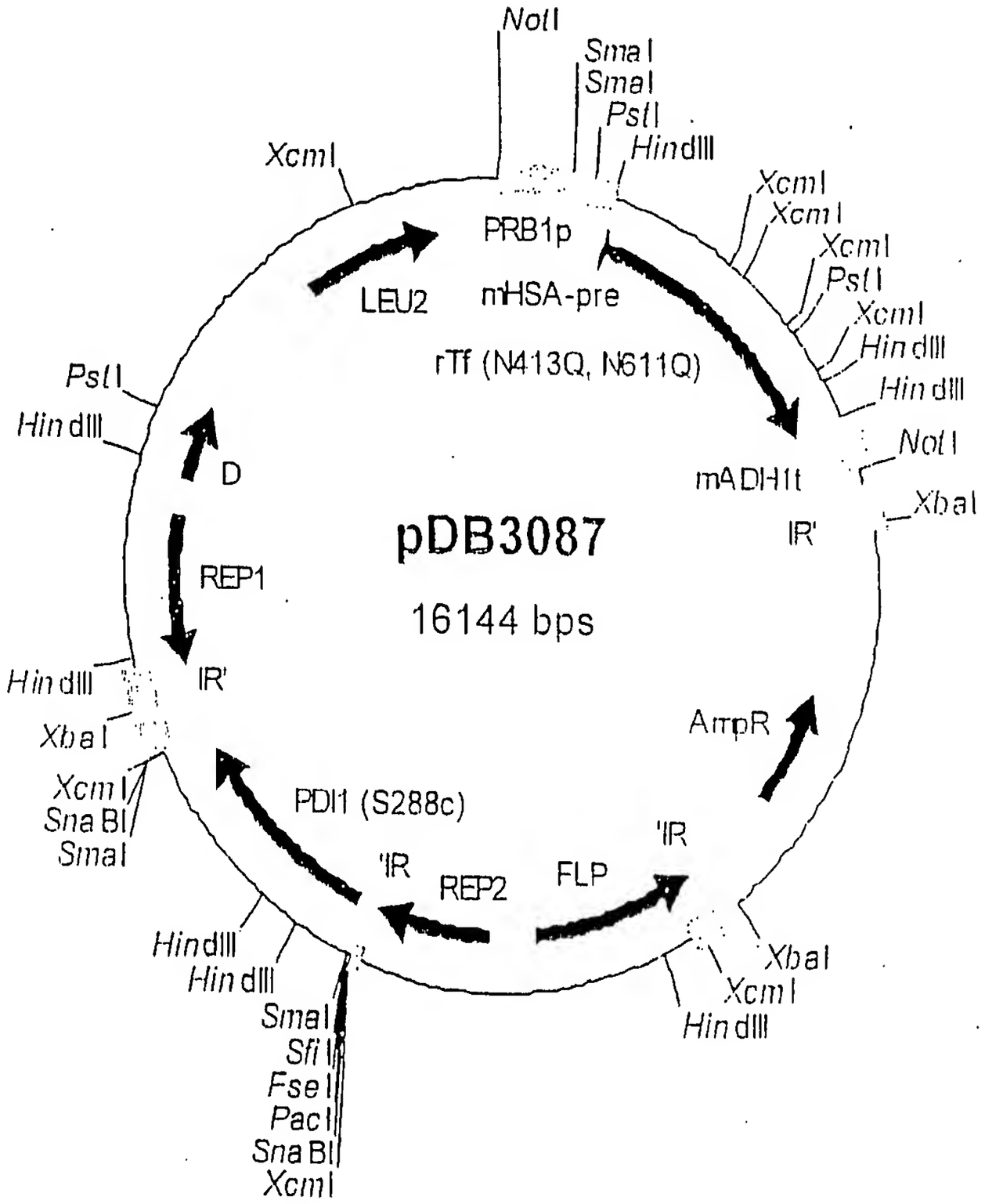
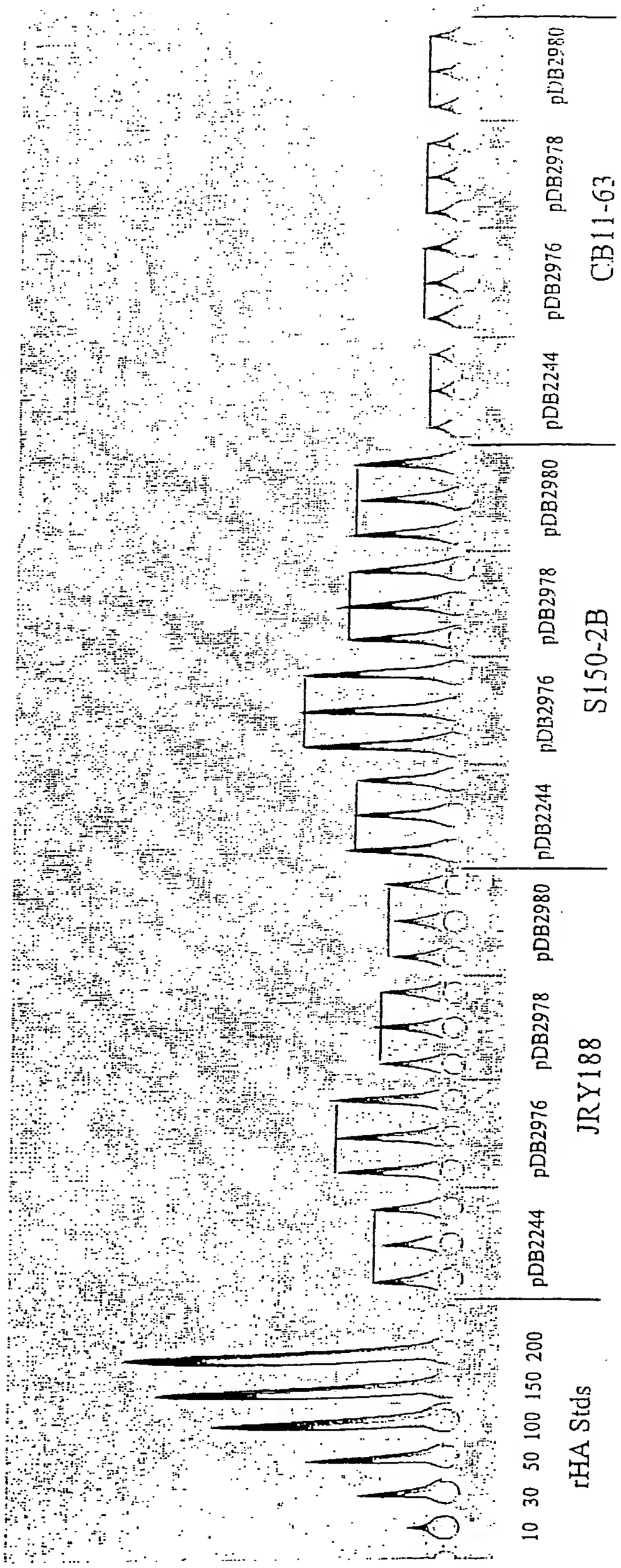


FIGURE 52

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pDB2978
pDB2980
JRY188
pDB2244
pDB2976
pDB2978
pDB2980
S150-2B
pDB2244
pDB2976
pDB2978
pDB2980
CB11-63
10 30 50 100 150 200
rHA Stds

FIGURE 53

FIGURE 54

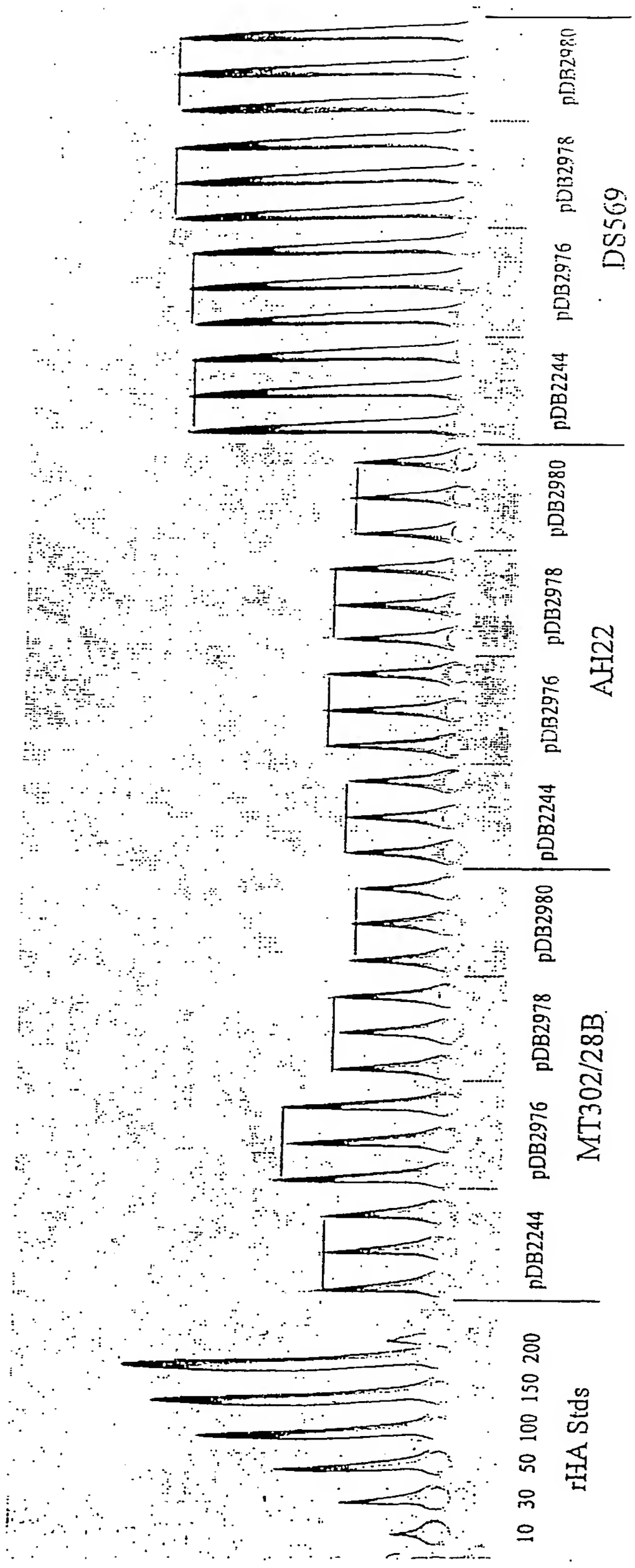
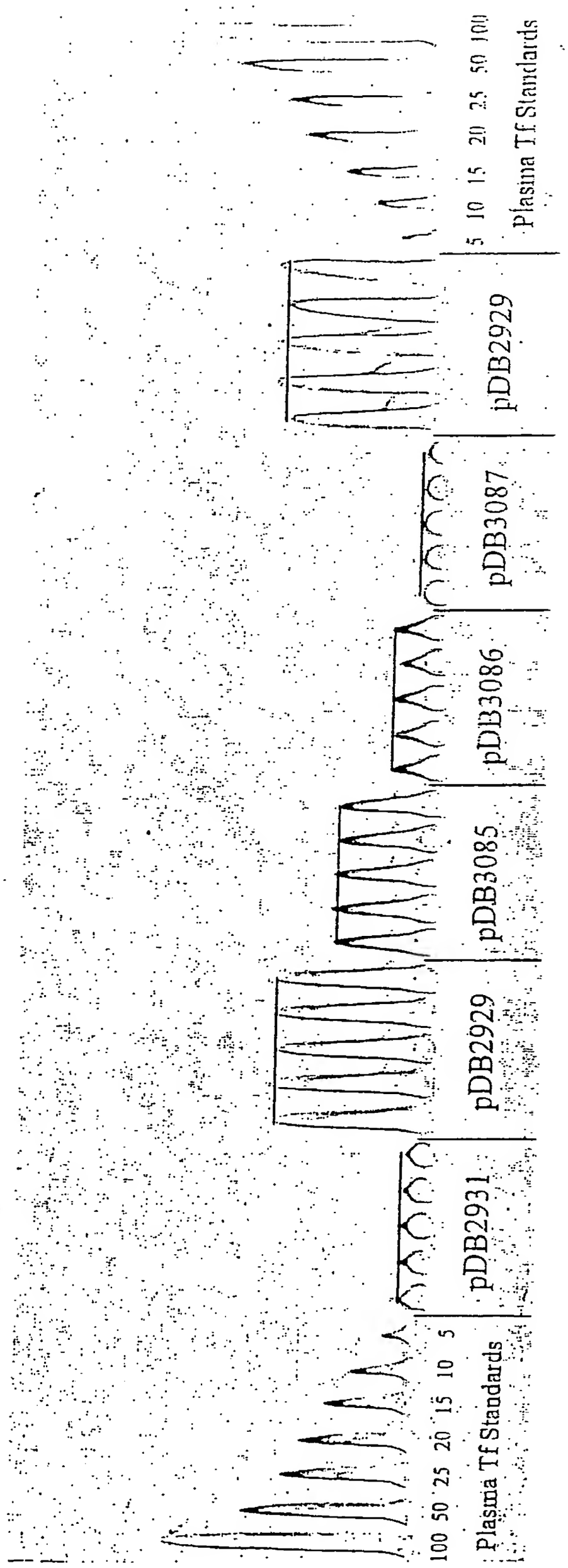

pDB2980
pDB2978
pDB2976
DS569
pDB2980
pDB2978
pDB2976
pDB2244
AH22
pDB2244
pDB2976
pDB2978
MT302/28B
10 30 50 100 150 200
rHA Stds

FIGURE 55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5 10 15 20 25 50 100
Plasma Tf Standards
pDB2929
pDB3085
pDB3086
pDB3087
100 50 25 20 15 10 5
Plasma Tf Standards
pDB2931

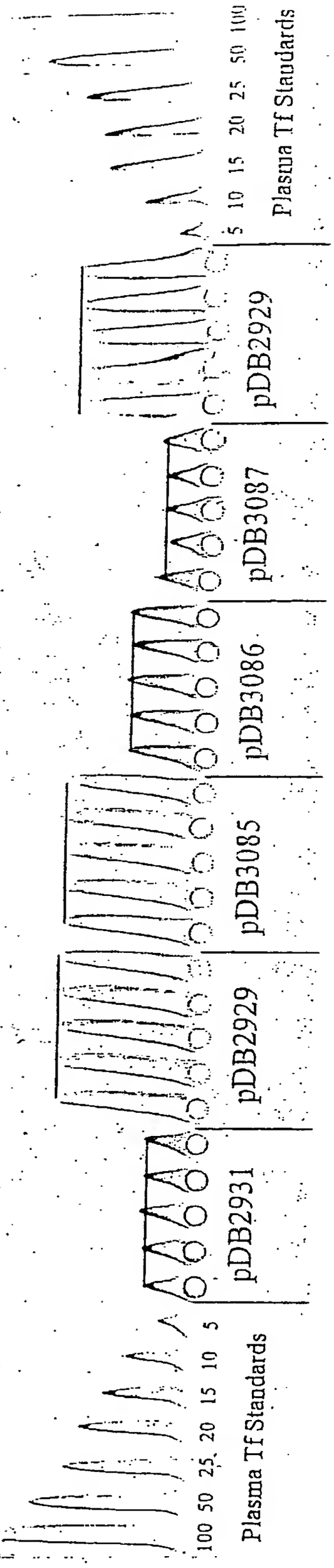

pDB2929
5 10 15 20 25 50 100
Plasma Tf Standards
pDB2929
pDB3085
pDB3086
pDB3087
pDB2931
100 50 25 20 15 10 5
Plasma Tf Standards

Figure 56

FIGURE 58

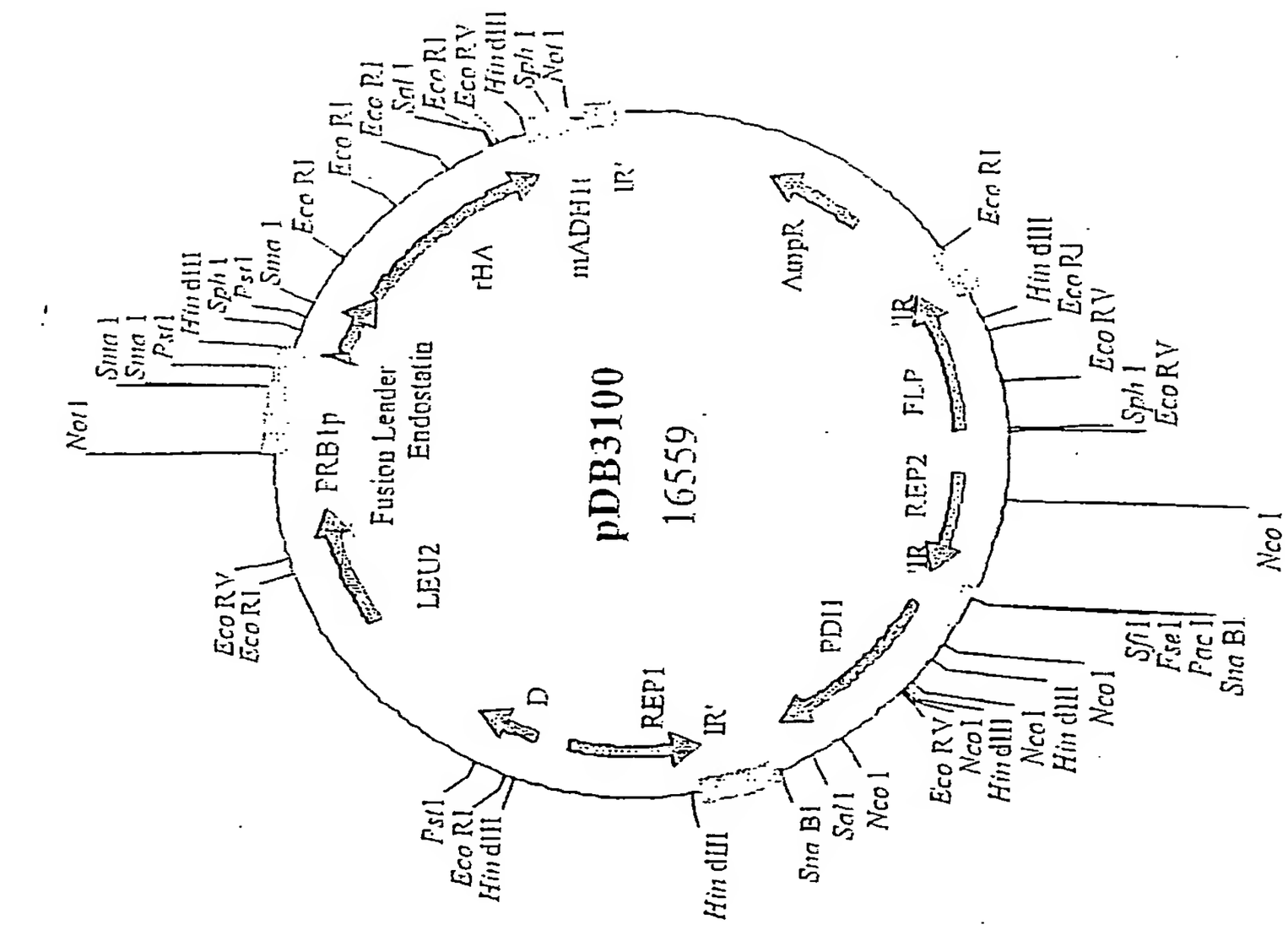


FIGURE 57

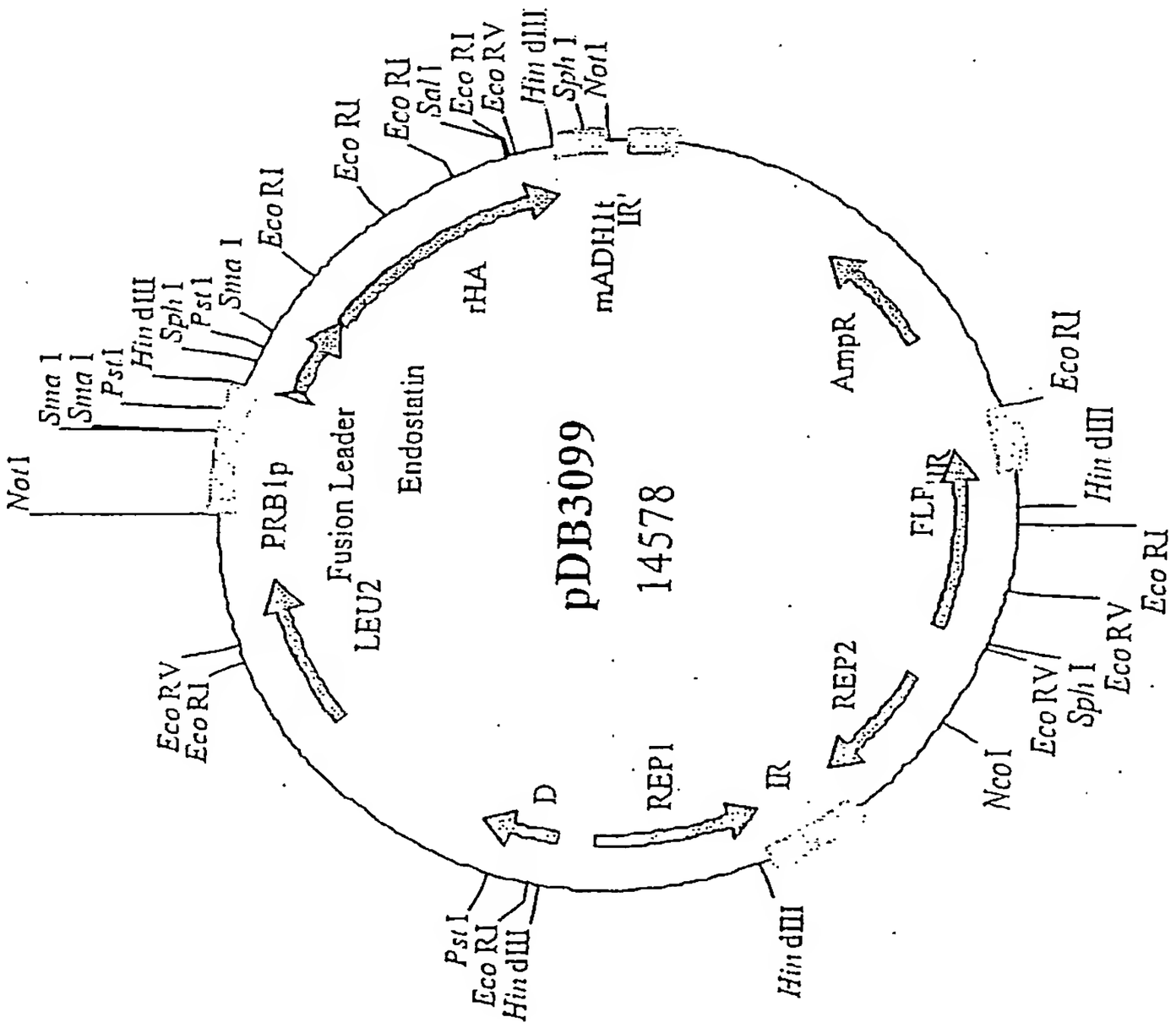


FIGURE 60

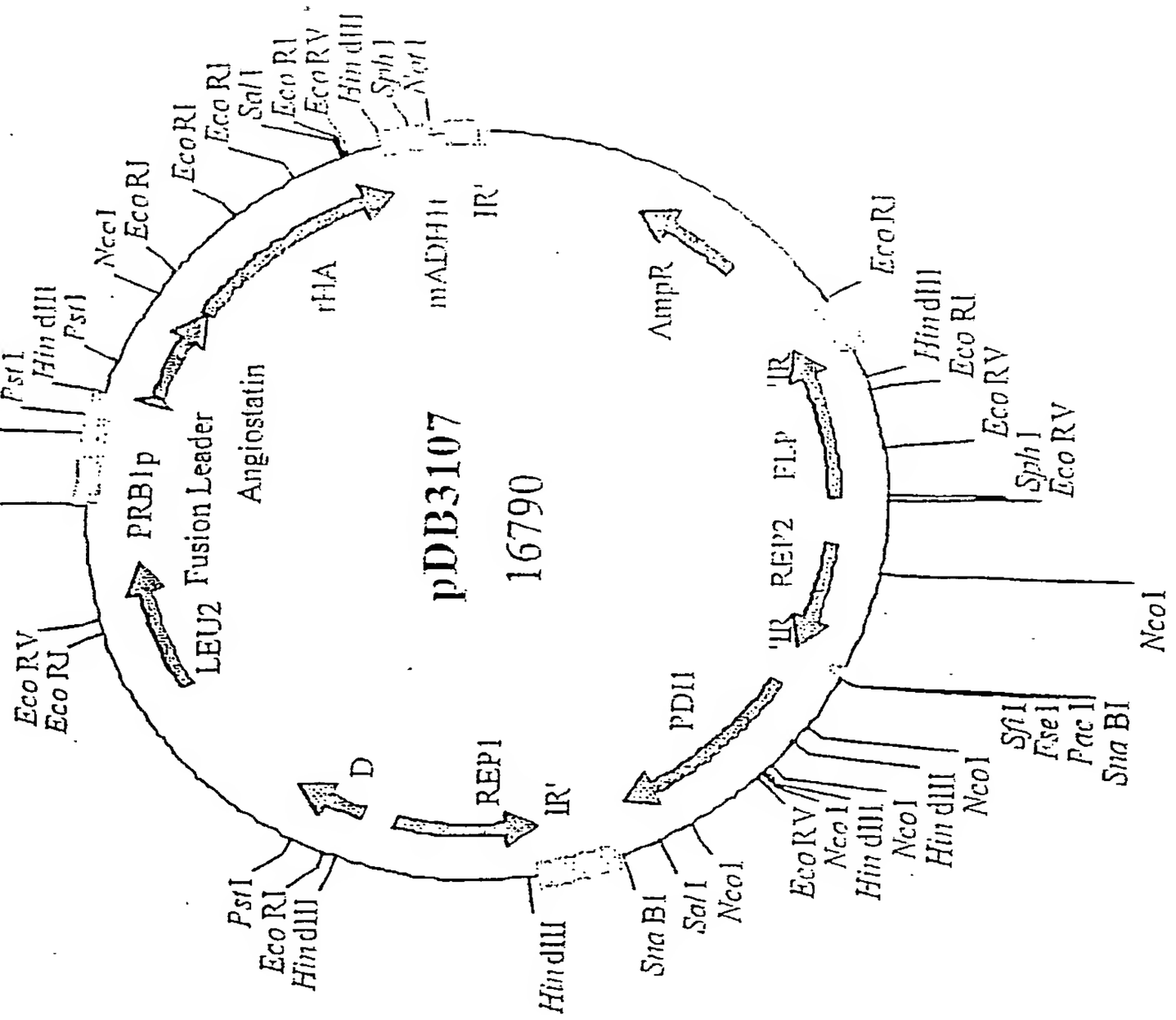


FIGURE 59

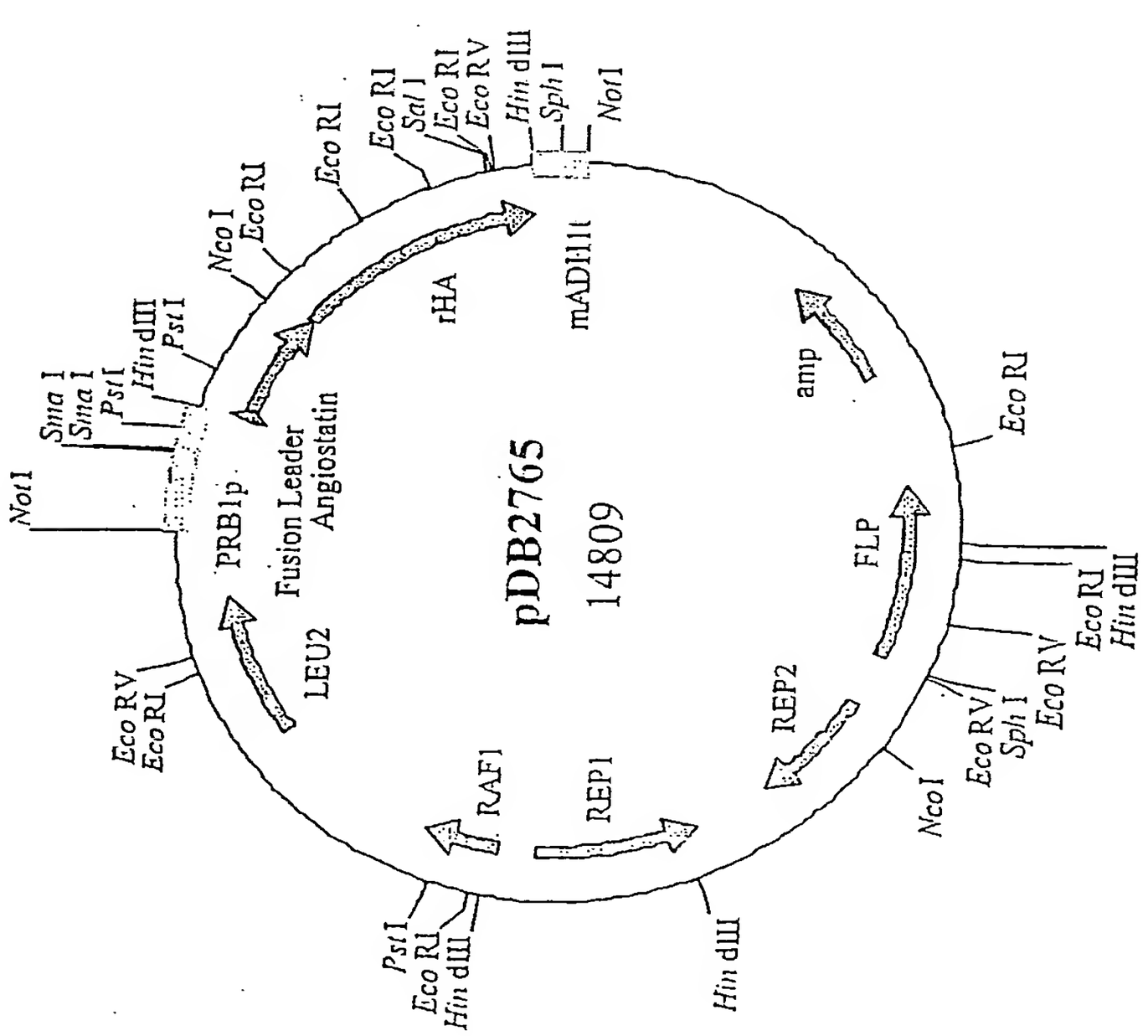


FIGURE 62

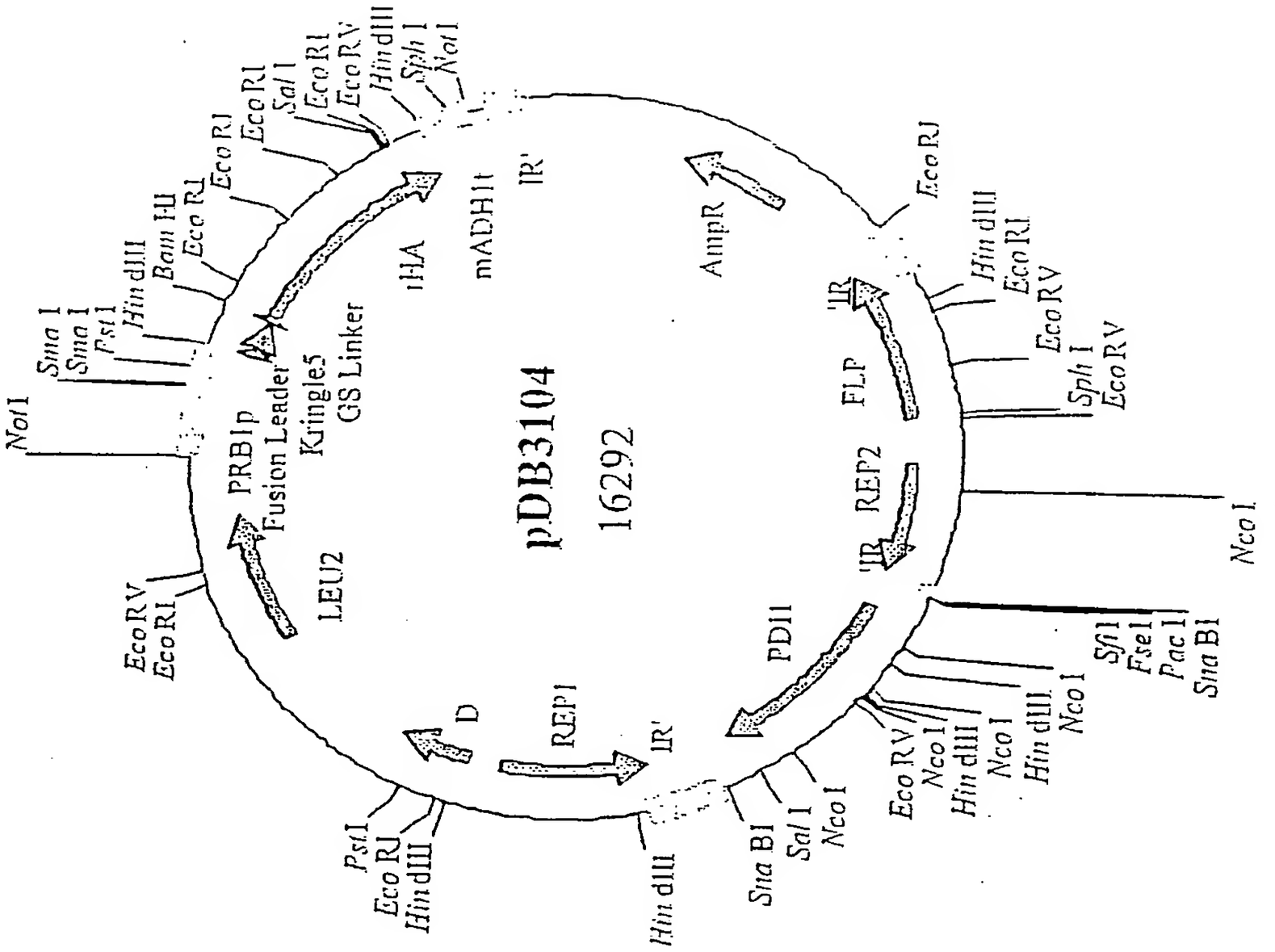


FIGURE 61

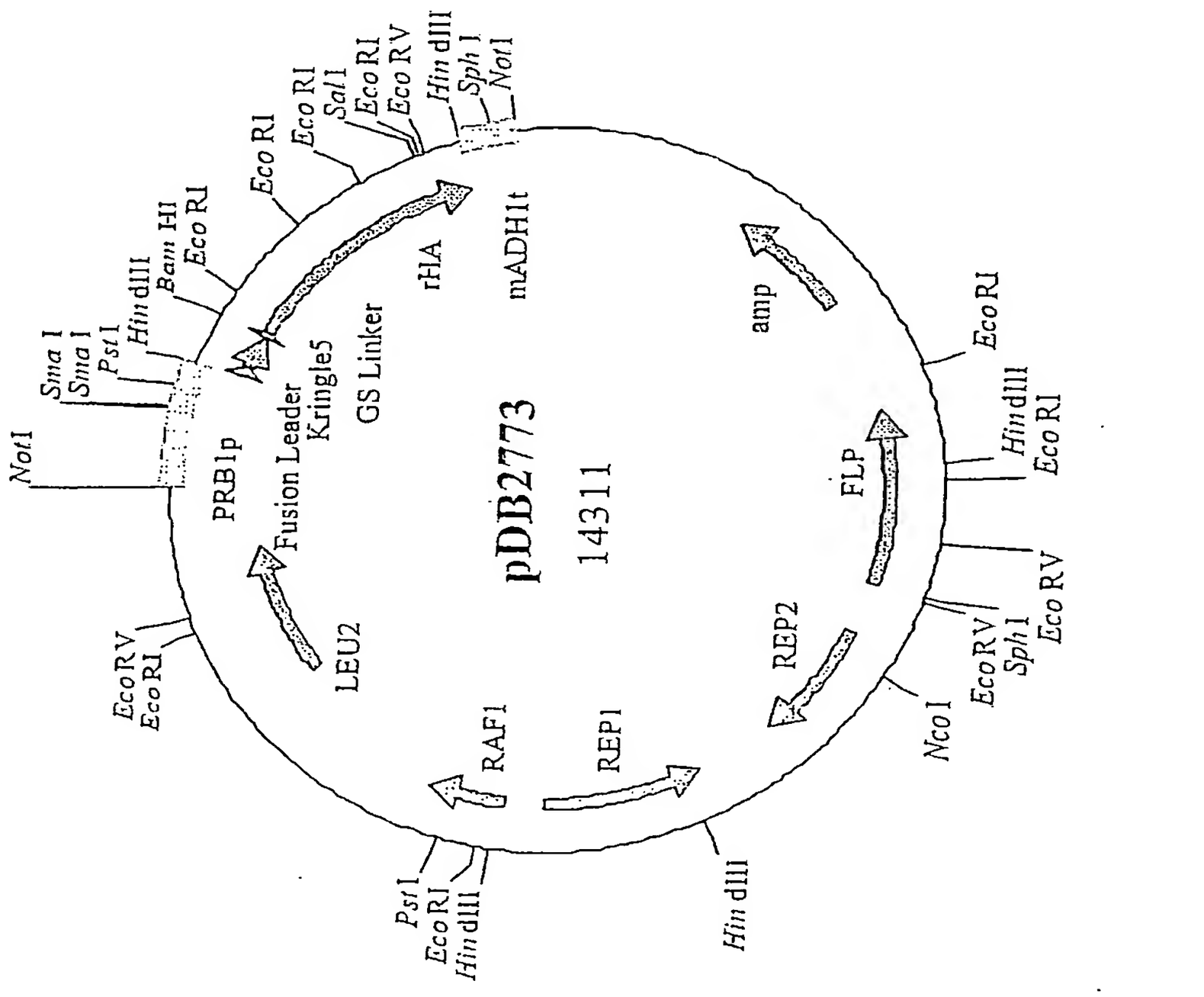


FIGURE 64

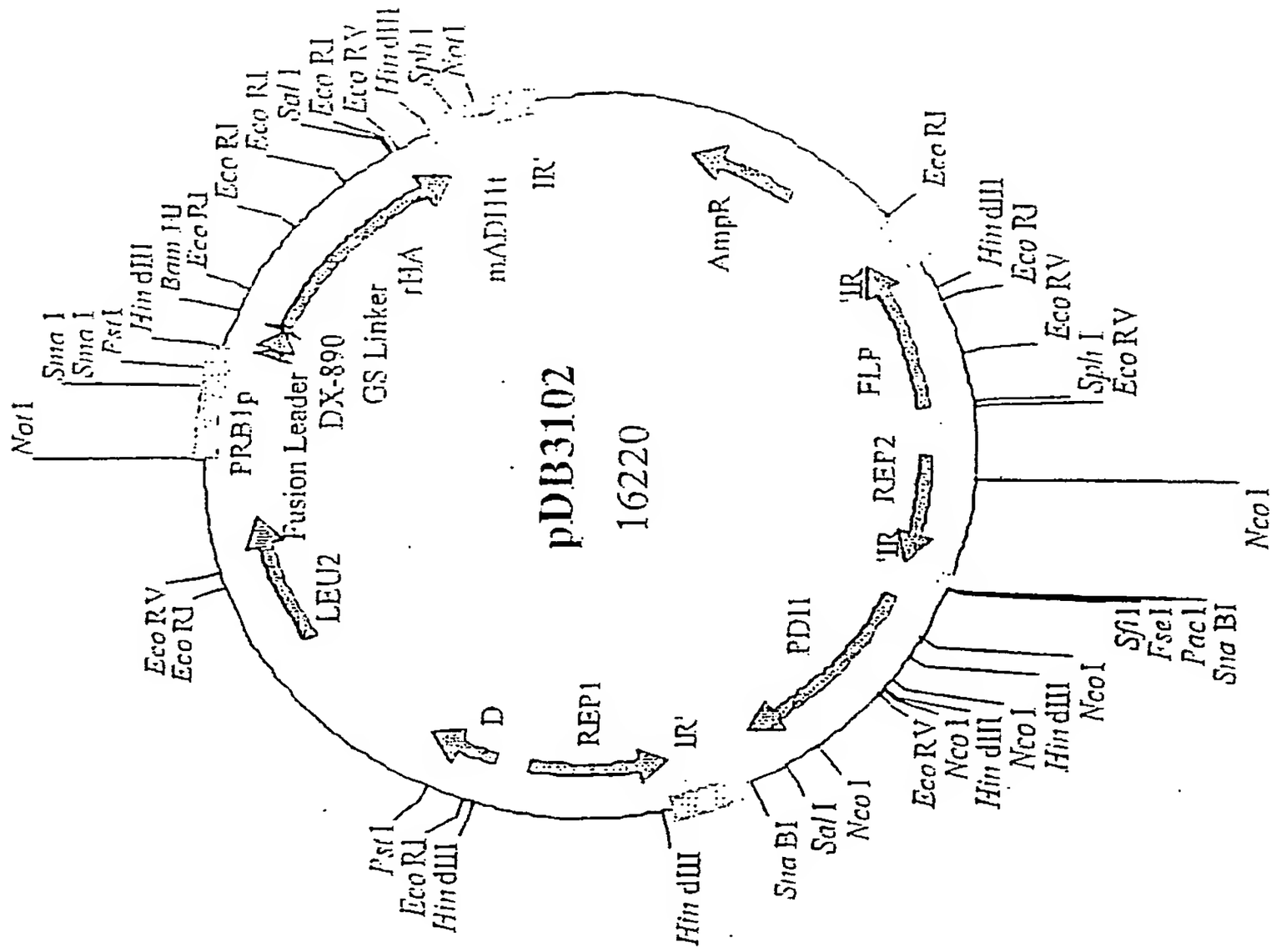


FIGURE 63

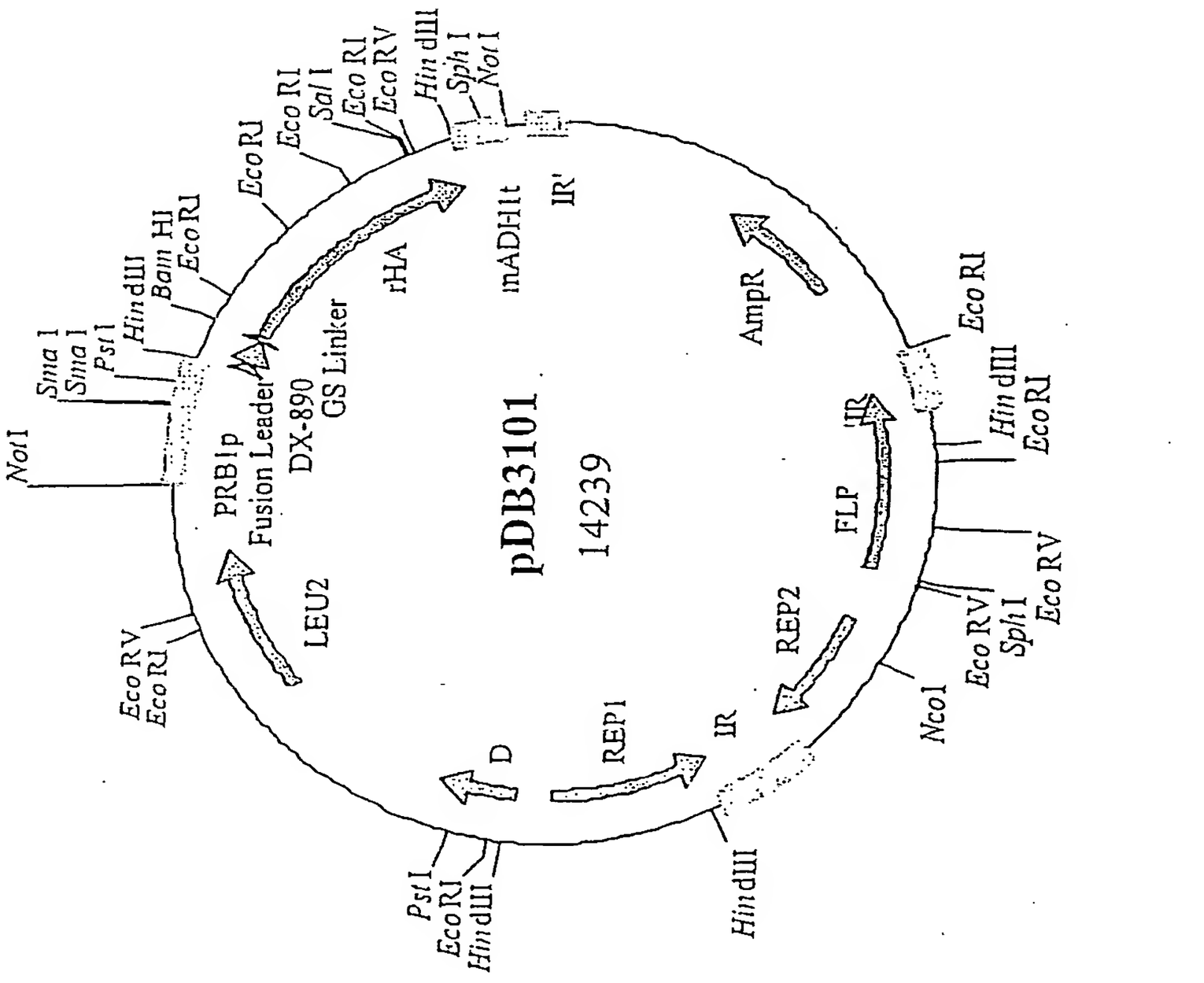


FIGURE 66

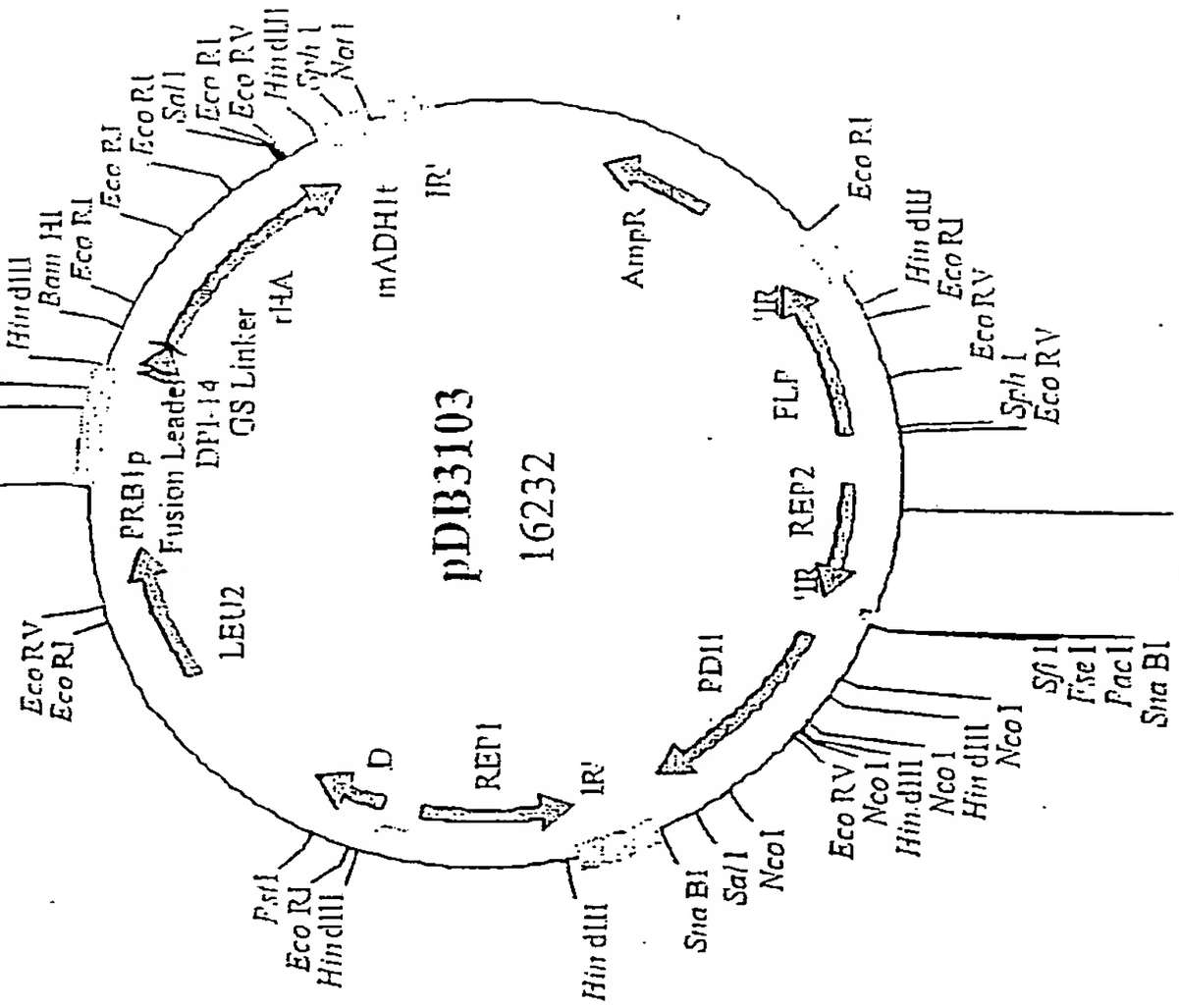


FIGURE 65

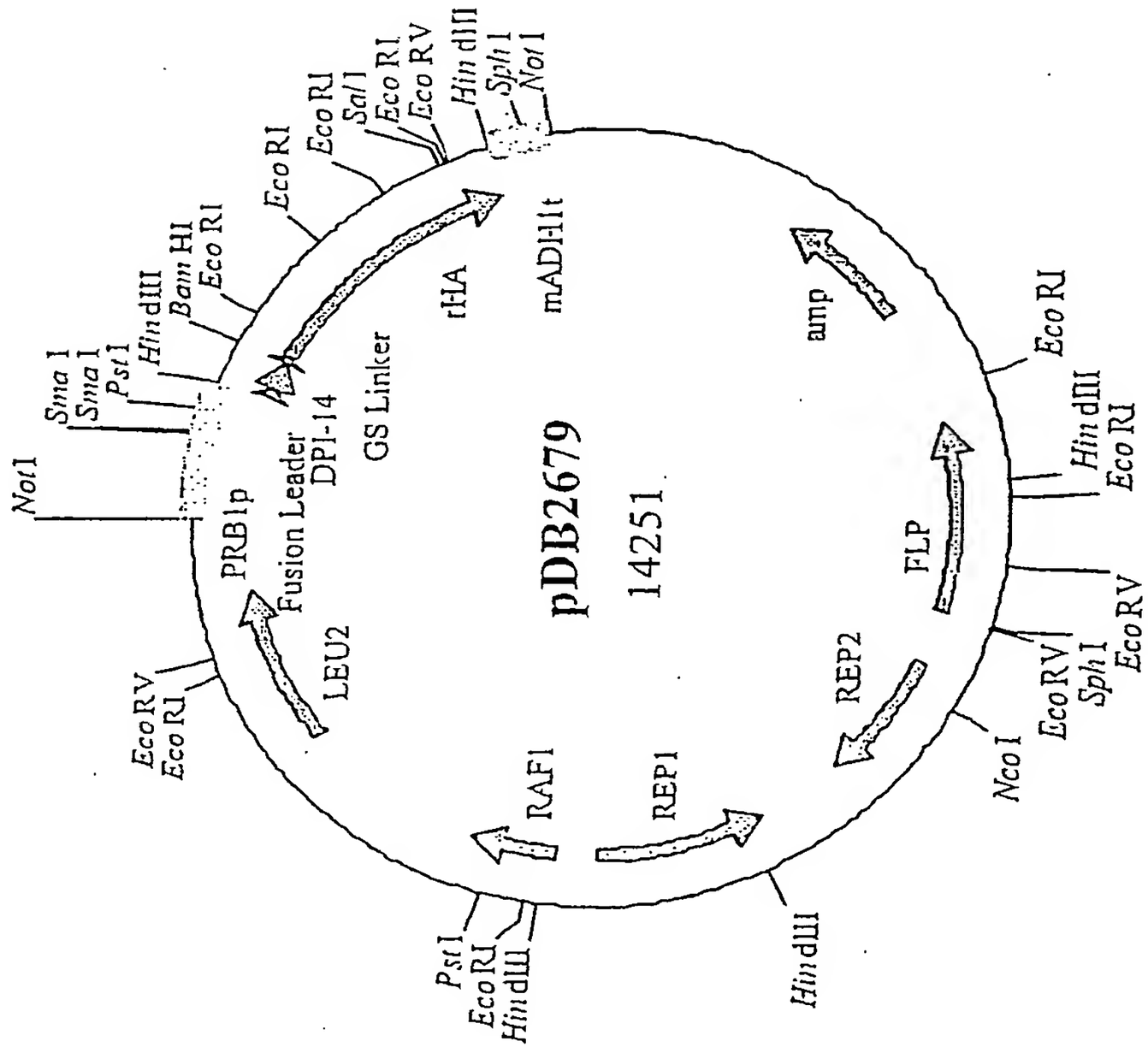


FIGURE 68

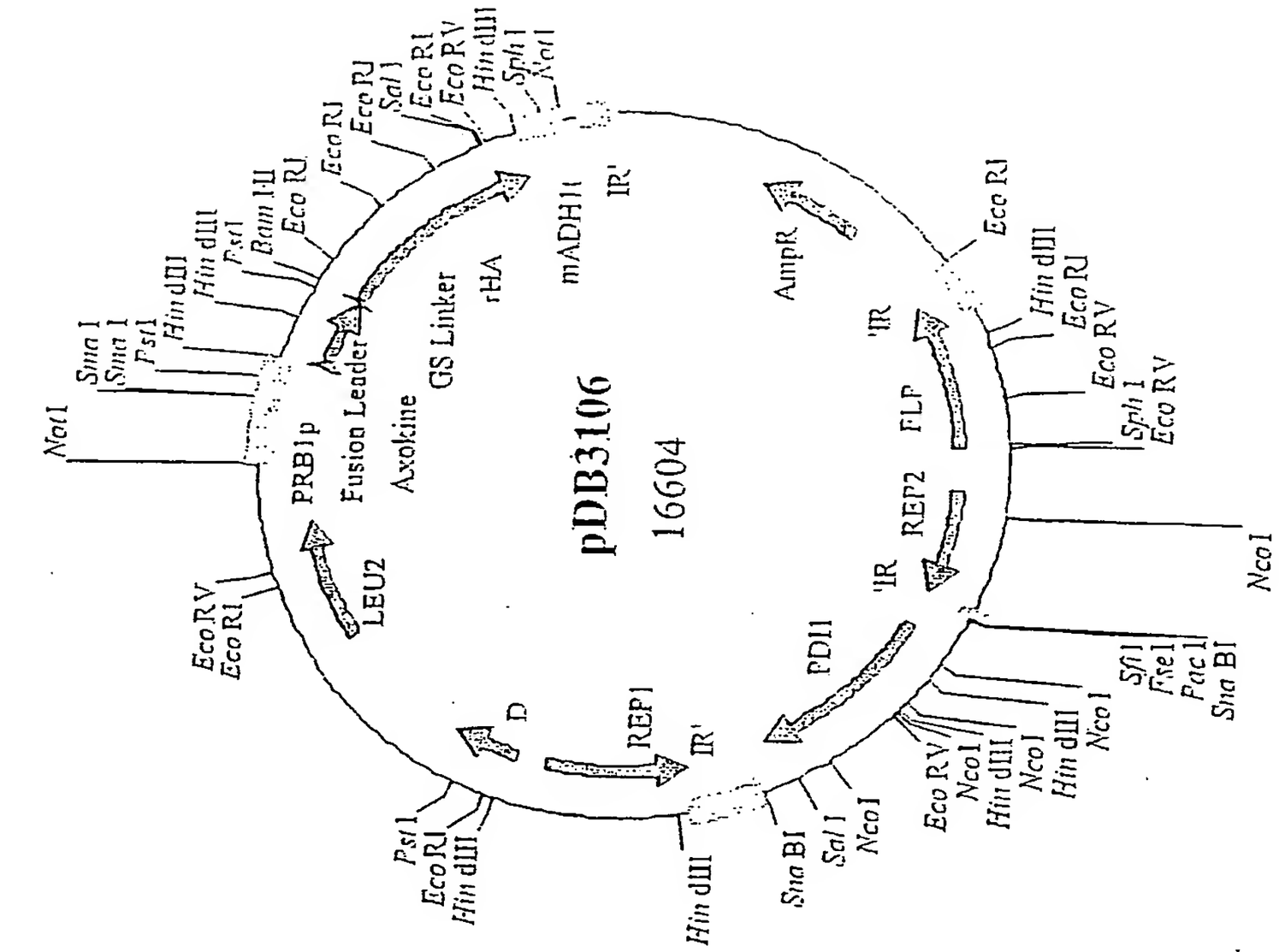


FIGURE 67

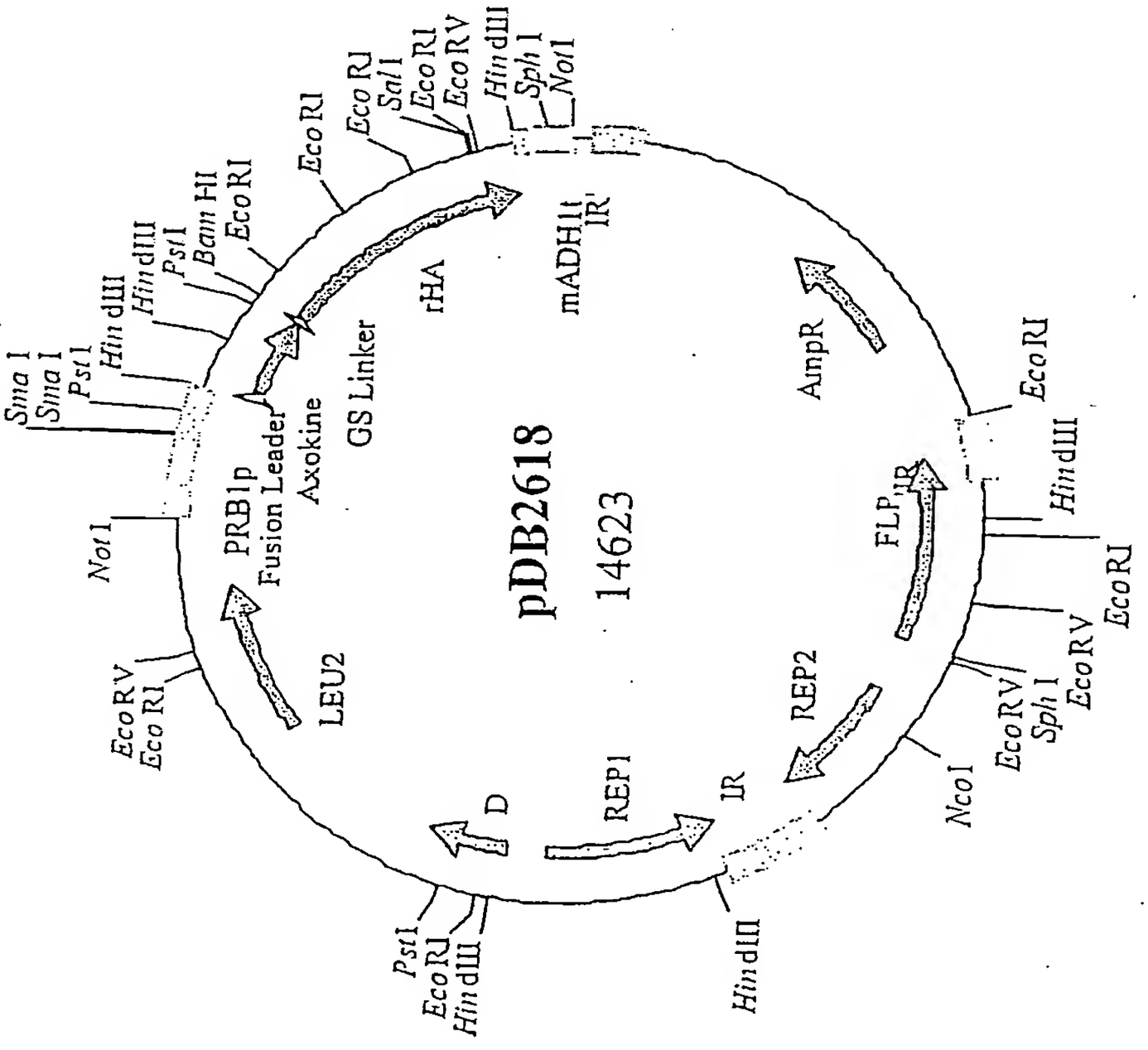


FIGURE 70

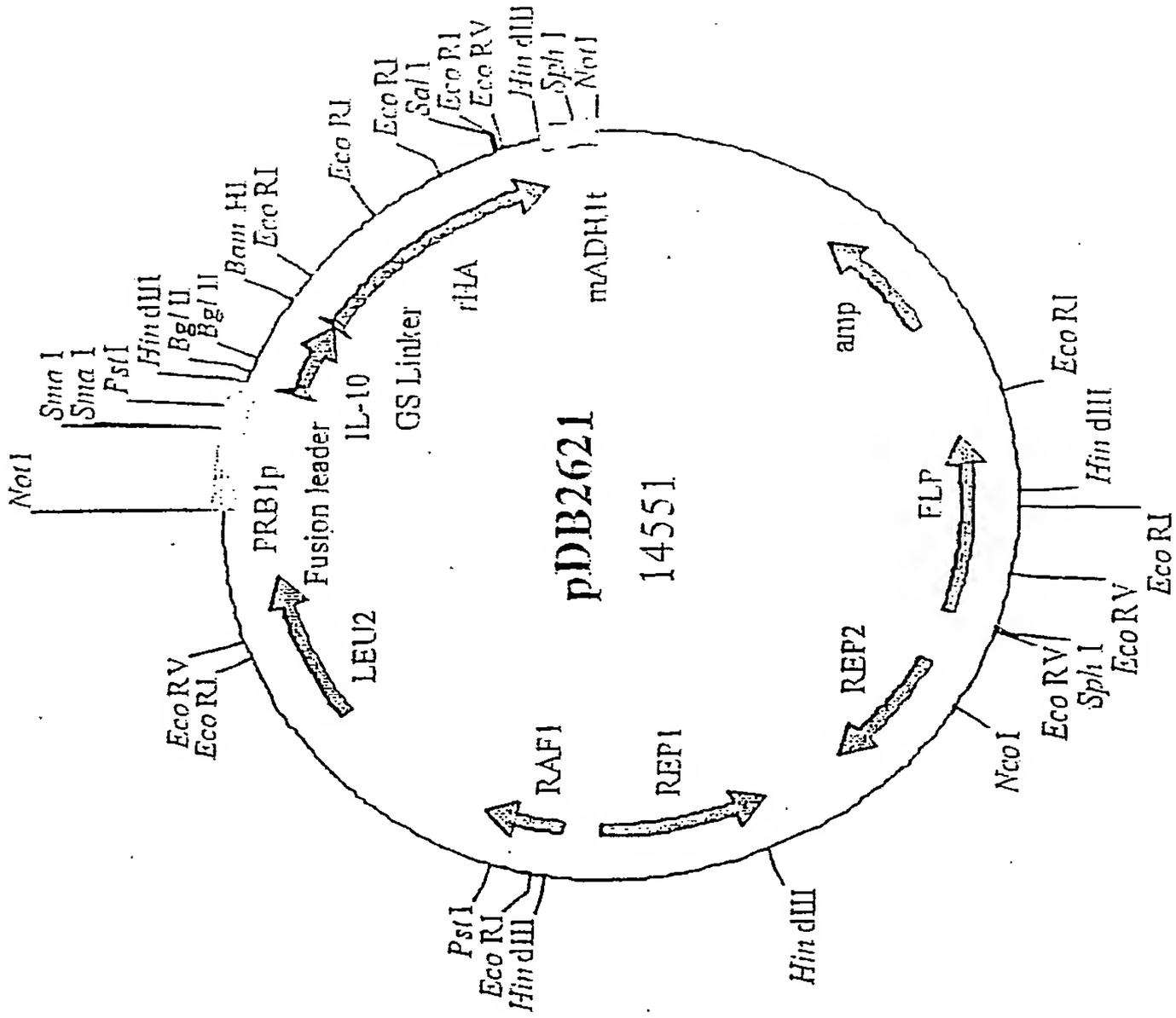
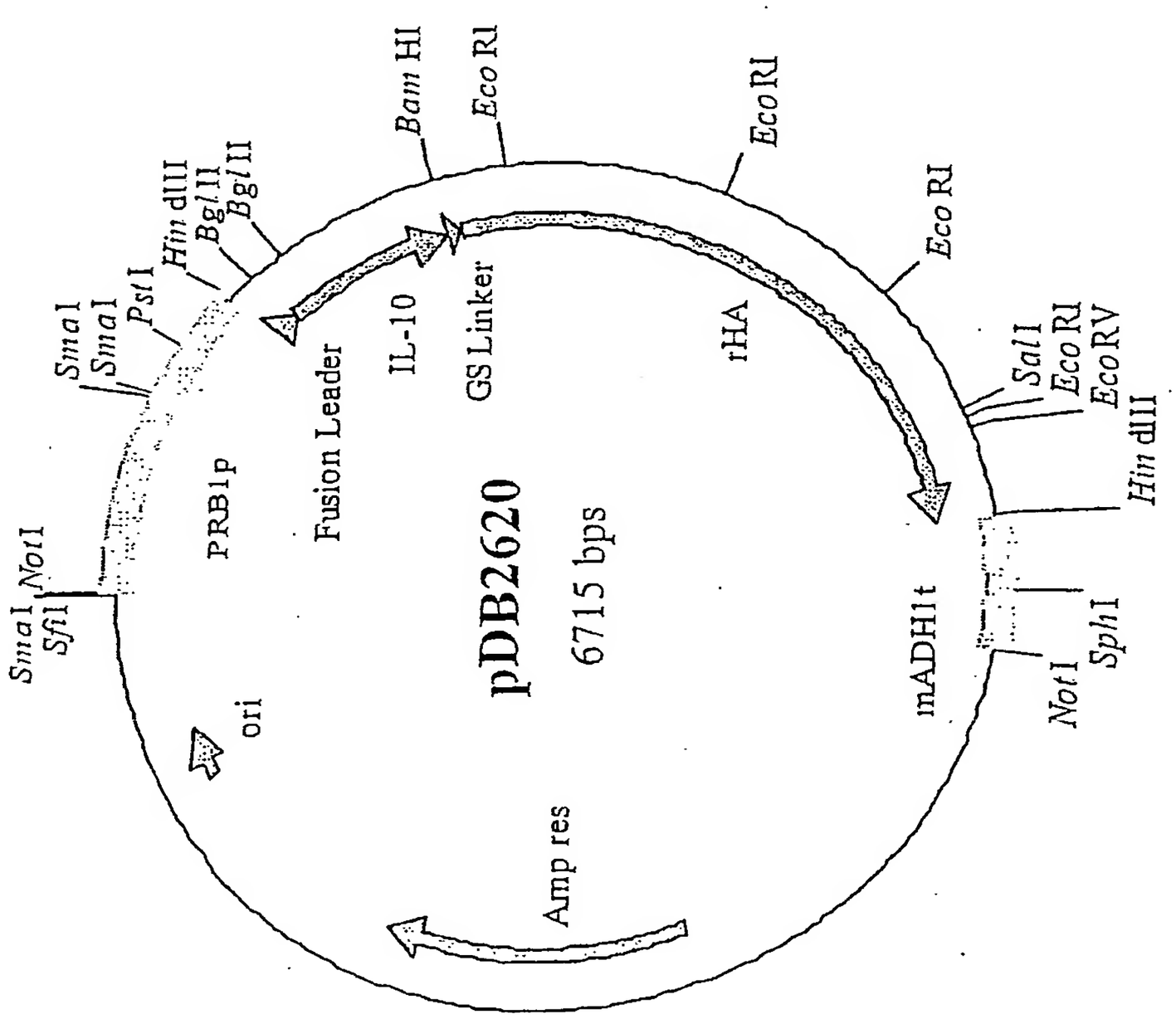


FIGURE 69



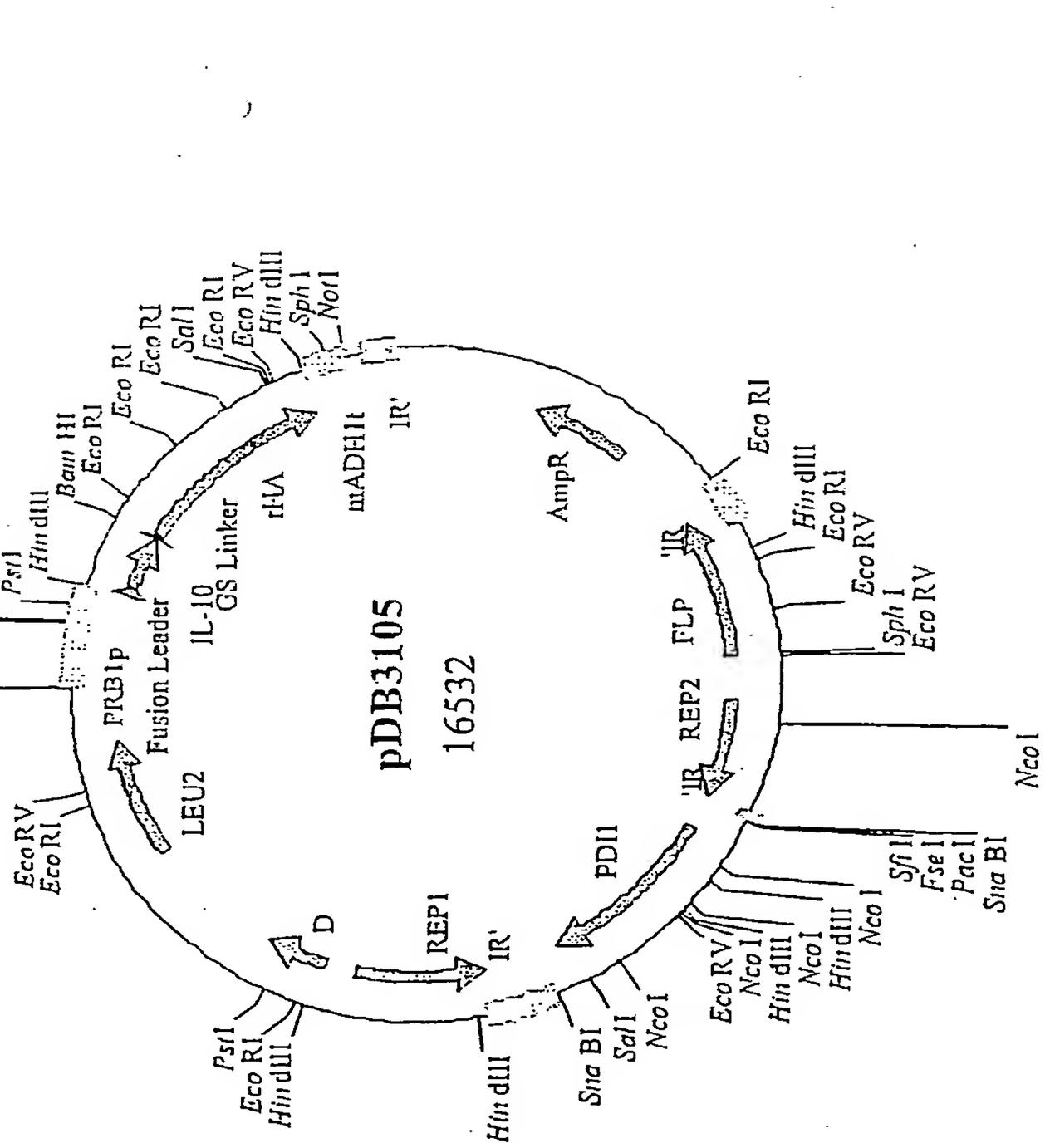
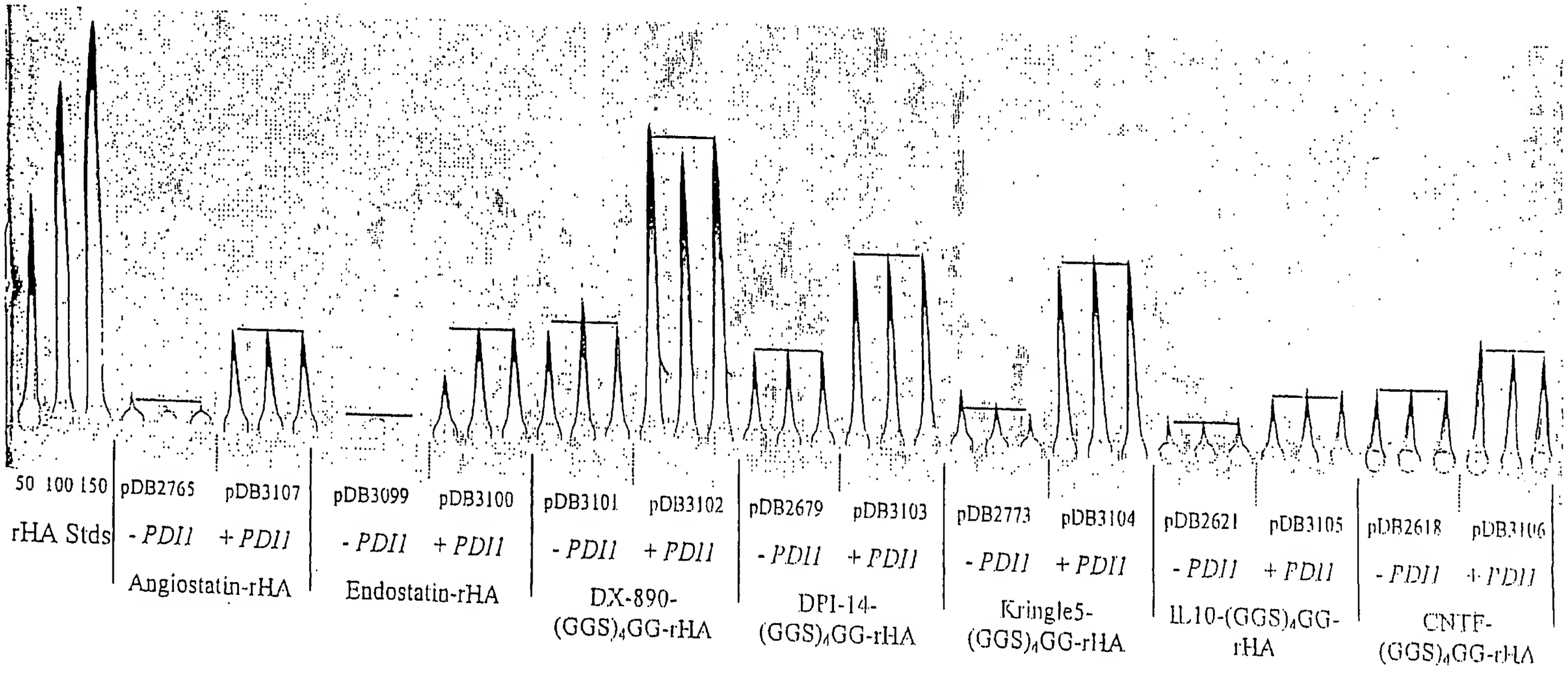

pDB3105
16532
Nor1
Sma 1
Sma 1
Pst1
Hin dIII
Bam HI
Eco RI
Eco RI
Eco RI
Sal I
Eco RI
Eco RV
Hin dIII
Sph 1
Nor1
PRB1p
Fusion Leader
IL-10
GS Linker
rHA
mADH1t
IR'
AmpR
Eco RI
Hin dIII
Eco RI
Eco RV
Sph 1
Eco RV
Nco1
Sfi II
Fse 1
Pac1
Sna BI
Eco RV
Eco RI
LEU2
IR'
REP2
FLP
IR'
PD11
D
REP1
IR'
Psi1
Eco RI
Hin dIII
Hin dIII
Sna BI
Sal I
Nco I
Eco RV
Nco I
Hin dIII
Nco I
Hin dIII
Nco I

FIGURE 71

FIGURE 72


50 100 150
rHA Stds
pDB2765
pDB3107
- PDI1
+ PDI1
Angiostatin-rHA
pDB3099
pDB3100
- PDI1
+ PDI1
Endostatin-rHA
pDB3101
pDB3102
- PDI1
+ PDI1
DX-890-(GGS)4GG-rHA
pDB2679
pDB3103
- PDI1
+ PDI1
DPI-14-(GGS)4GG-rHA
pDB2773
pDB3104
- PDI1
+ PDI1
Kringle5-(GGS)4GG-rHA
pDB2621
pDB3105
- PDI1
+ PDI1
IL10-(GGS)4GG-rHA
pDB2618
pDB3106
- PDI1
+ PDI1
CNTF-(GGS)4GG-rHA

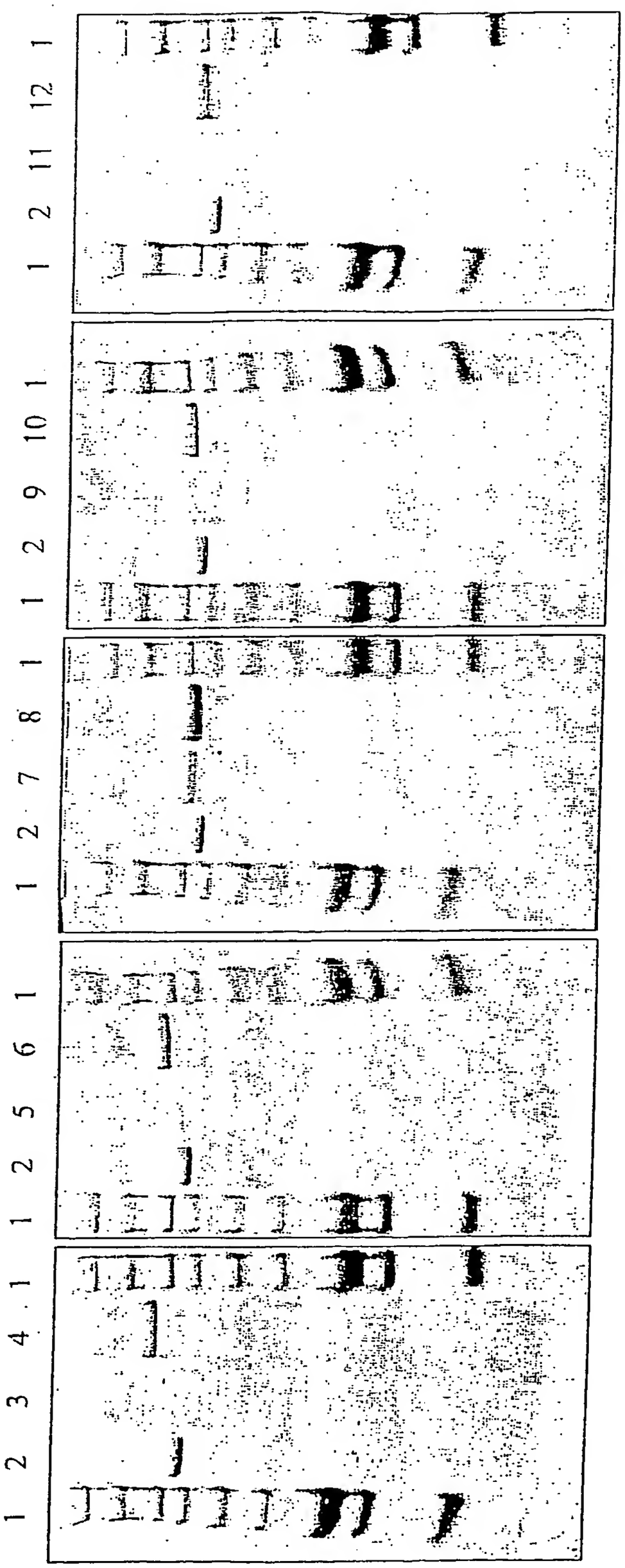


FIGURE 73

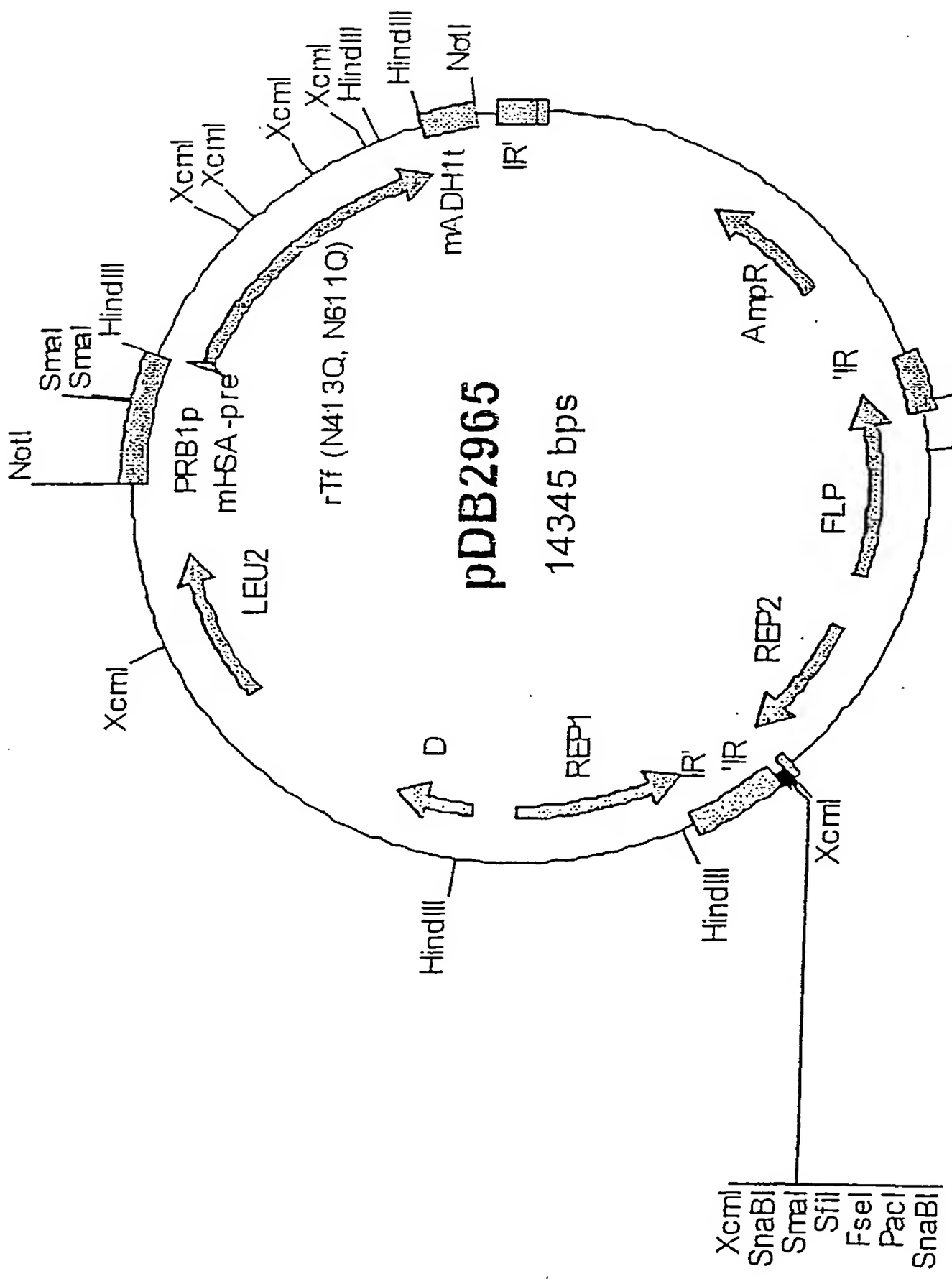

pDB2965
14345 bps
NotI
SmaI
SmaI
HindIII
PRB1p
mHSA-pre
rTf (N413Q, N611Q)
mADH1t
IR'
HindIII
NotI
XcmI
XcmI
XcmI
XcmI
HindIII
AmpR
'IR
XcmI
HindIII
REP2
FLP
'IR
REP1
R'
'IR
D
LEU2
XcmI
HindIII
HindIII
XcmI
XcmI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI

FIGURE 74

FIGURE 77

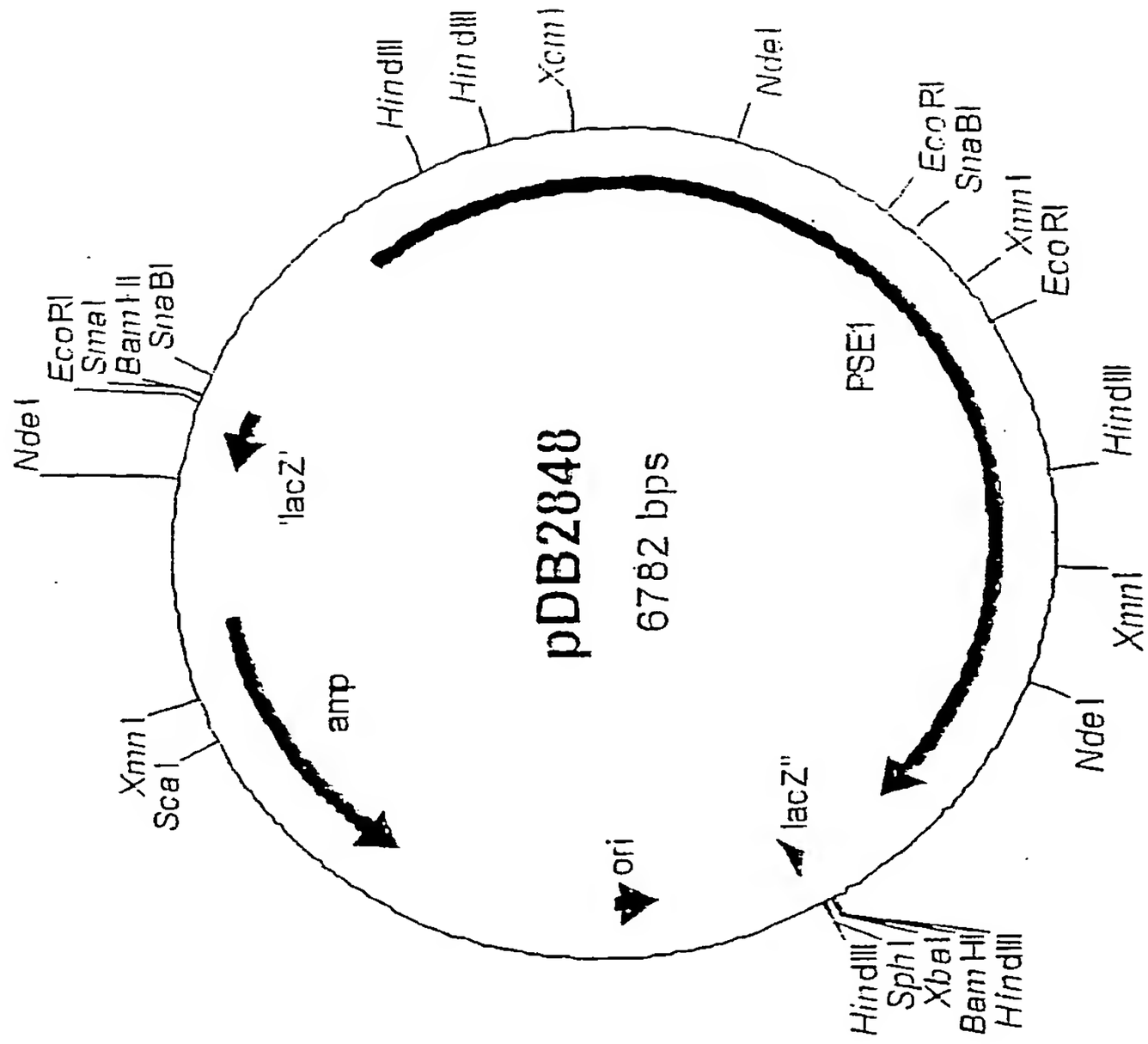
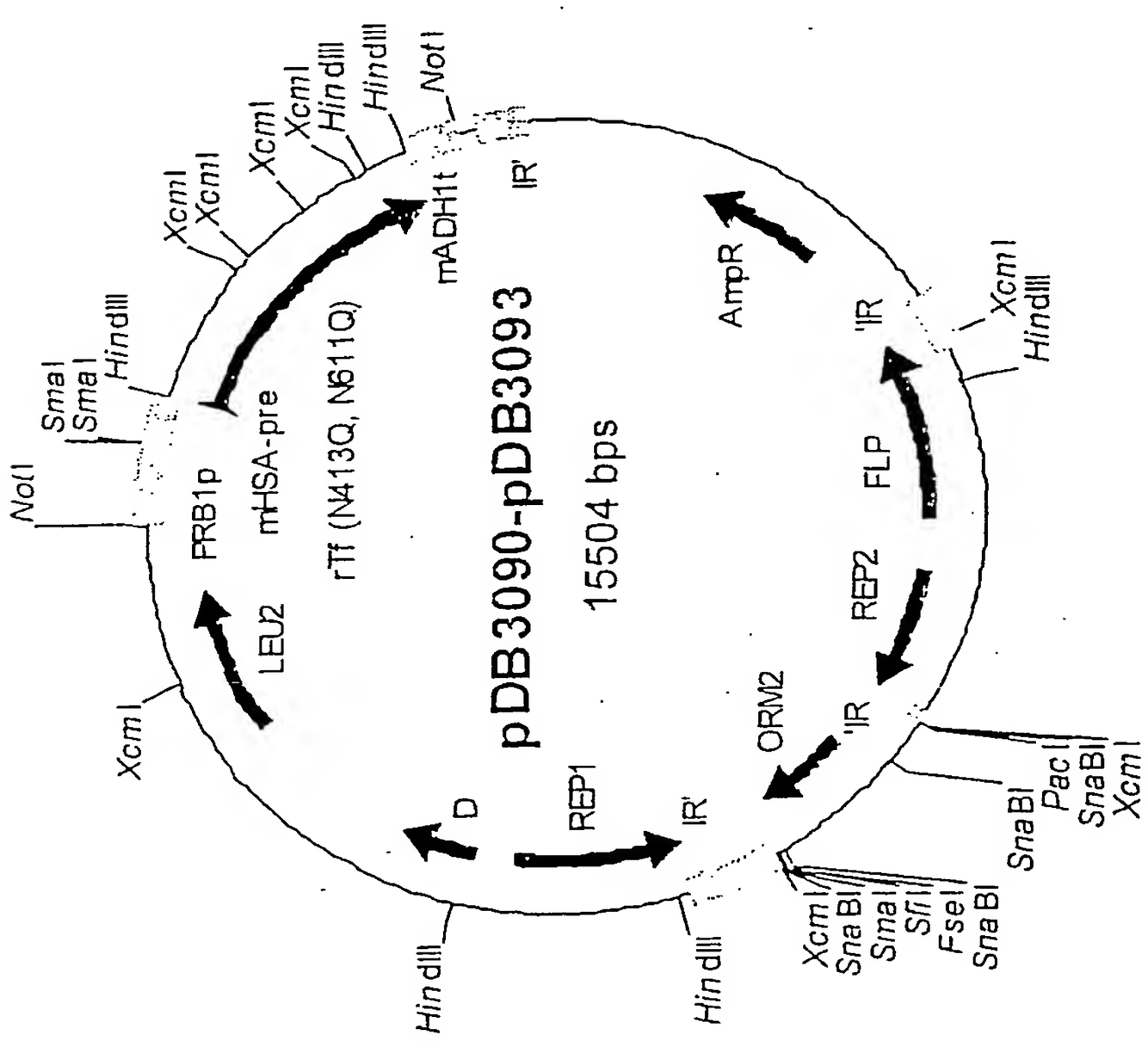


FIGURE 75



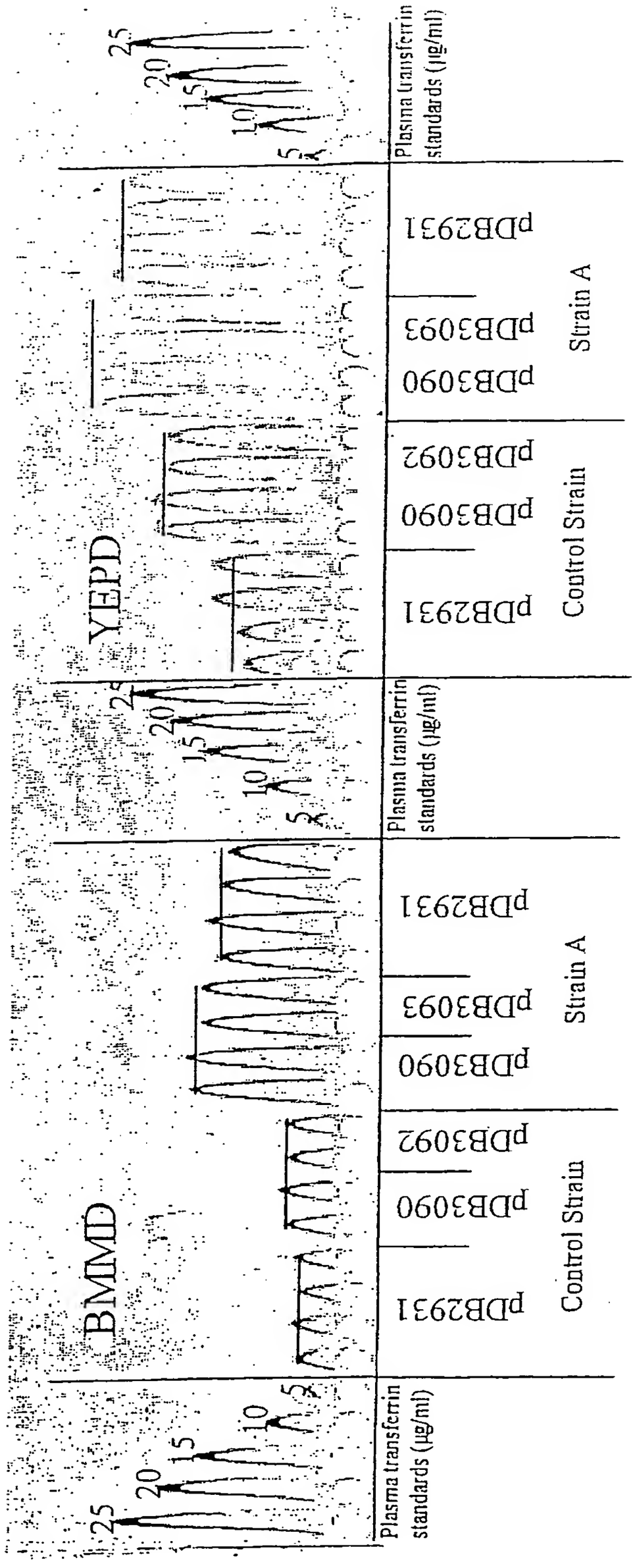

BMMD
YEPD
Plasma transferrin standards (µg/ml)
25
20
15
10
5
Control Strain
Strain A
pDB2931
pDB3090
pDB3092
pDB3093

Figure 76

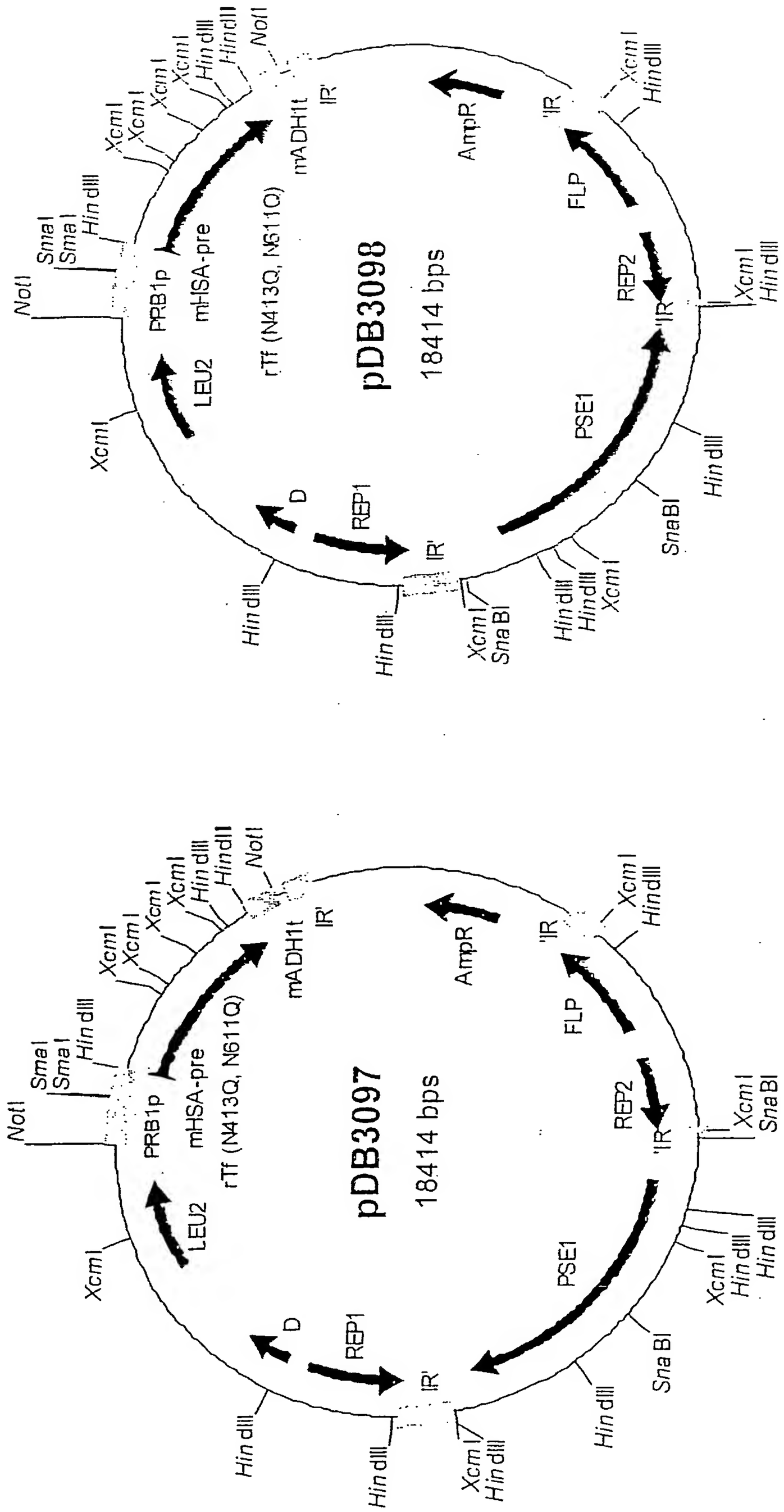


FIGURE 79

FIGURE 78

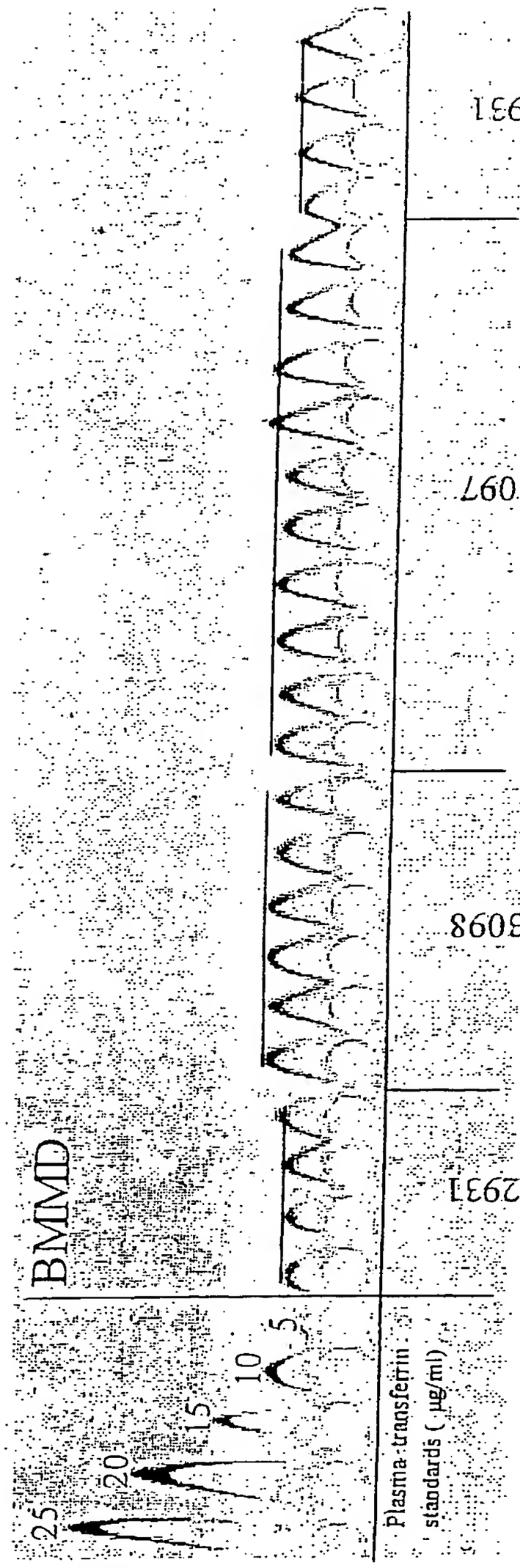

pDB2931
pDB3097
pDB3098
pDB2931
BMMD
25
20
15
10
5
1
Plasma transferrin standards (µg/ml)

Figure 80

FIGURE 82

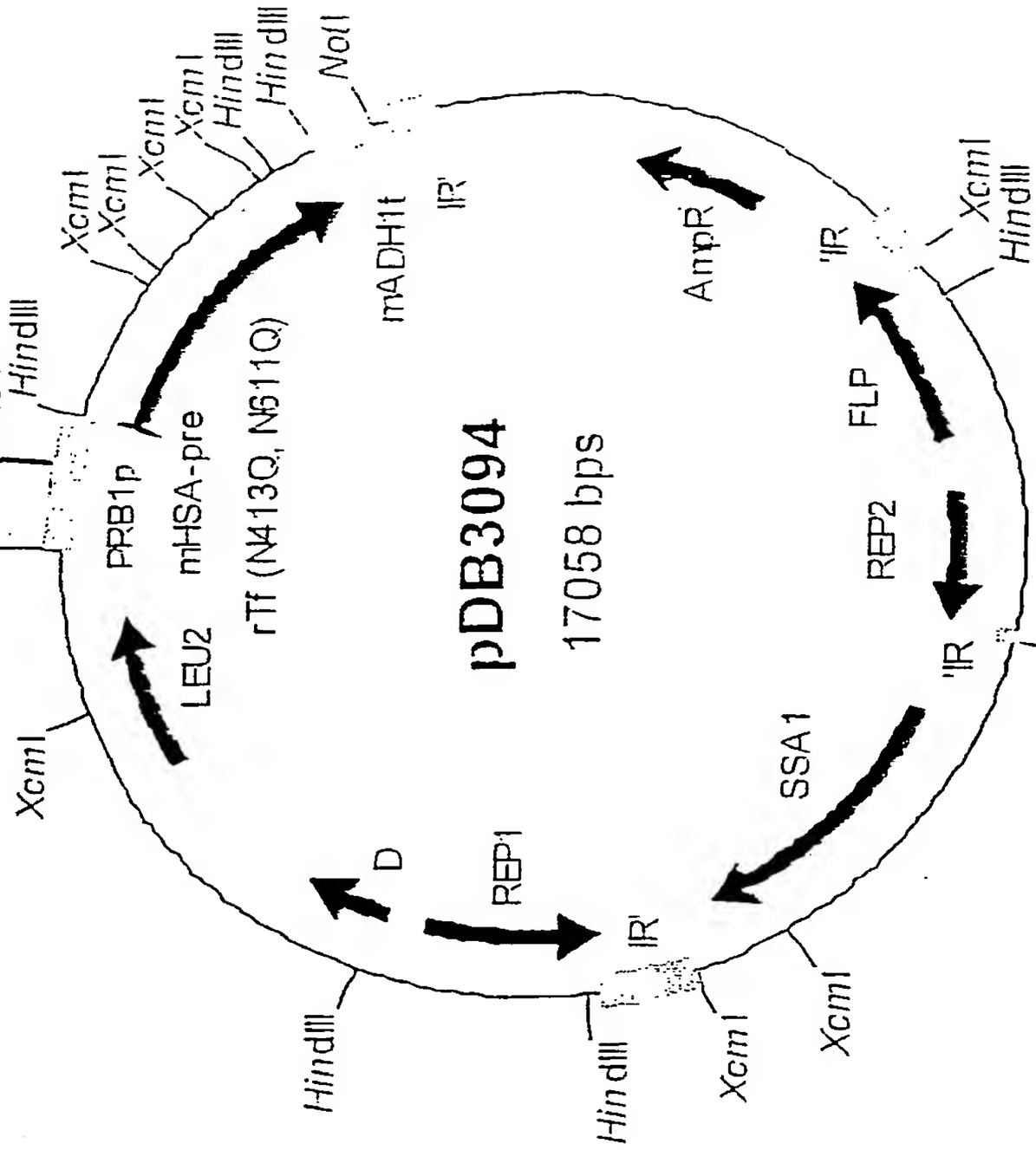


FIGURE 81

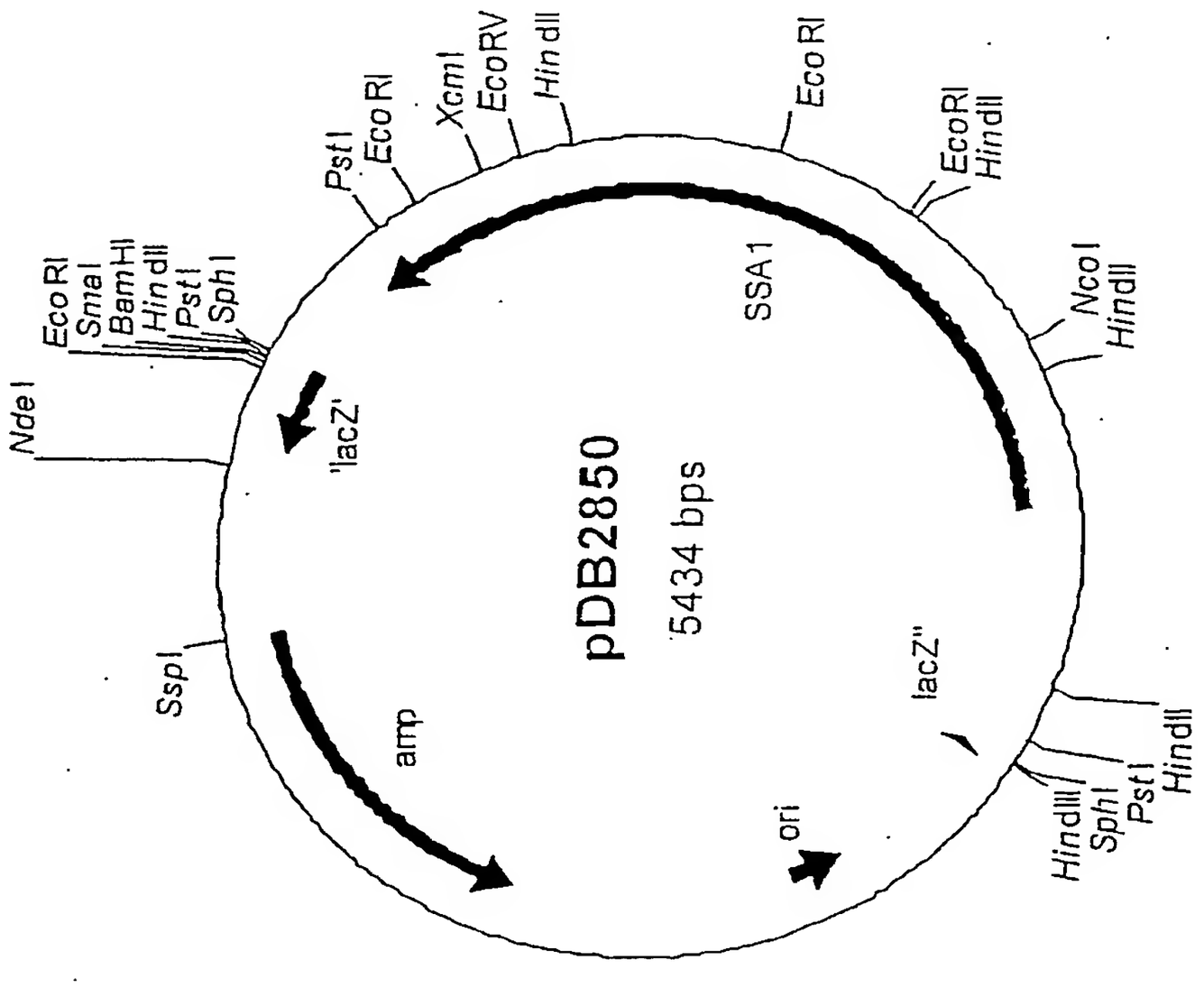
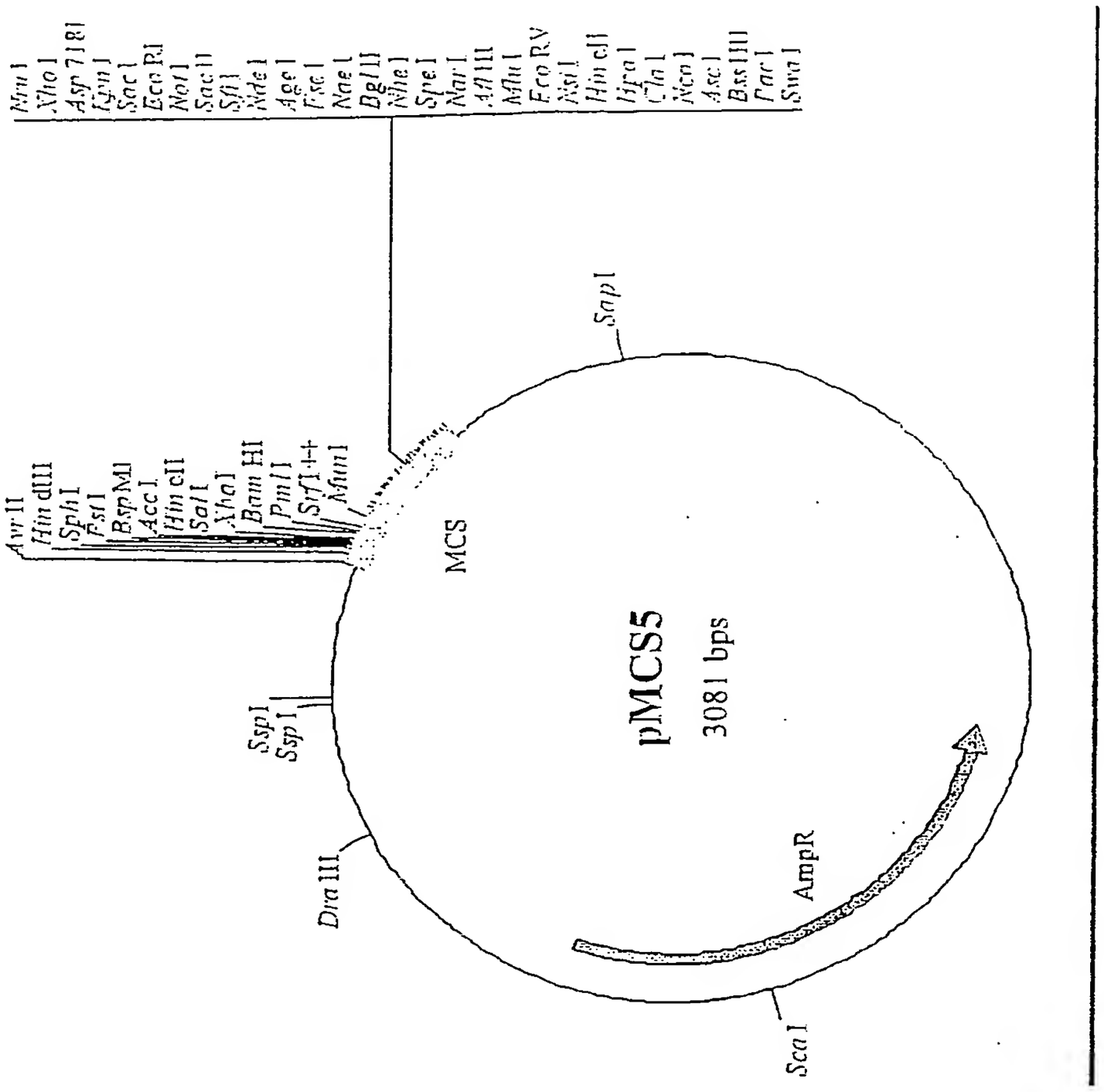


FIGURE 85



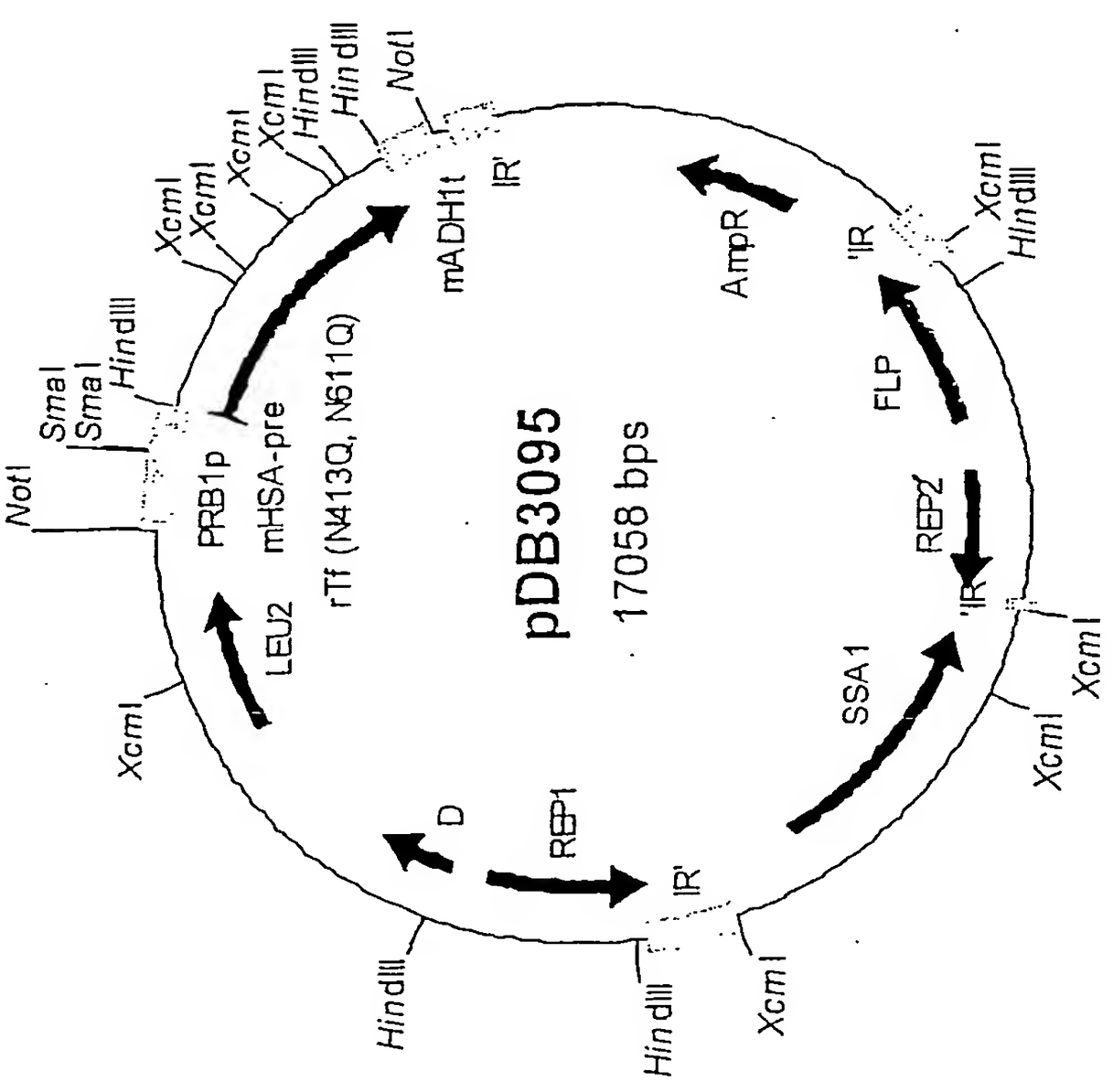


FIGURE 83

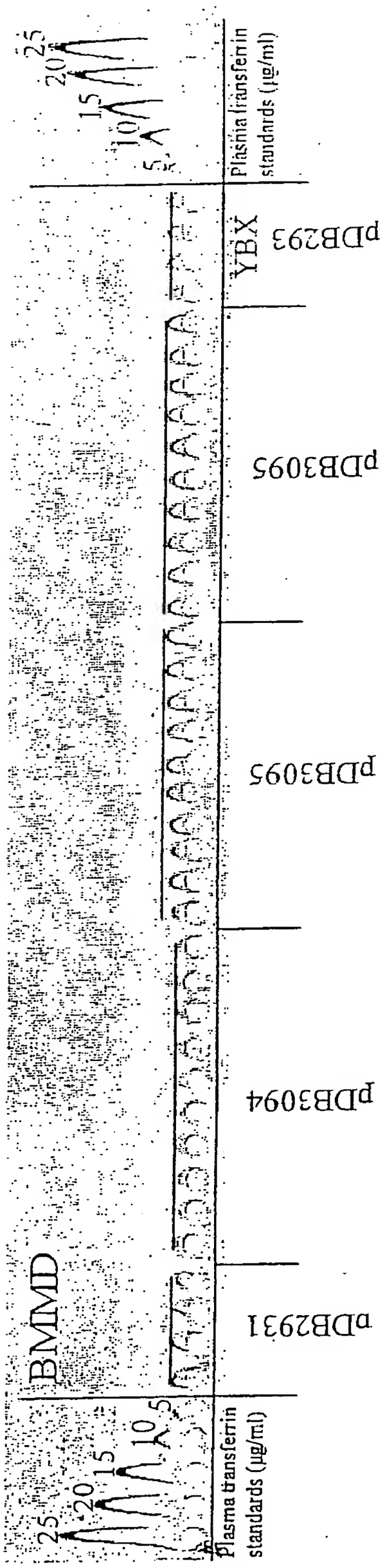

pDB2931
BMMD
Plasma transferrin standards (µg/ml)
25
20
15
10
5
pDB3094
pDB3095
pDB3095
YBX
pDB2931
Plasma transferrin standards (µg/ml)

Figure 84

FIGURE 86

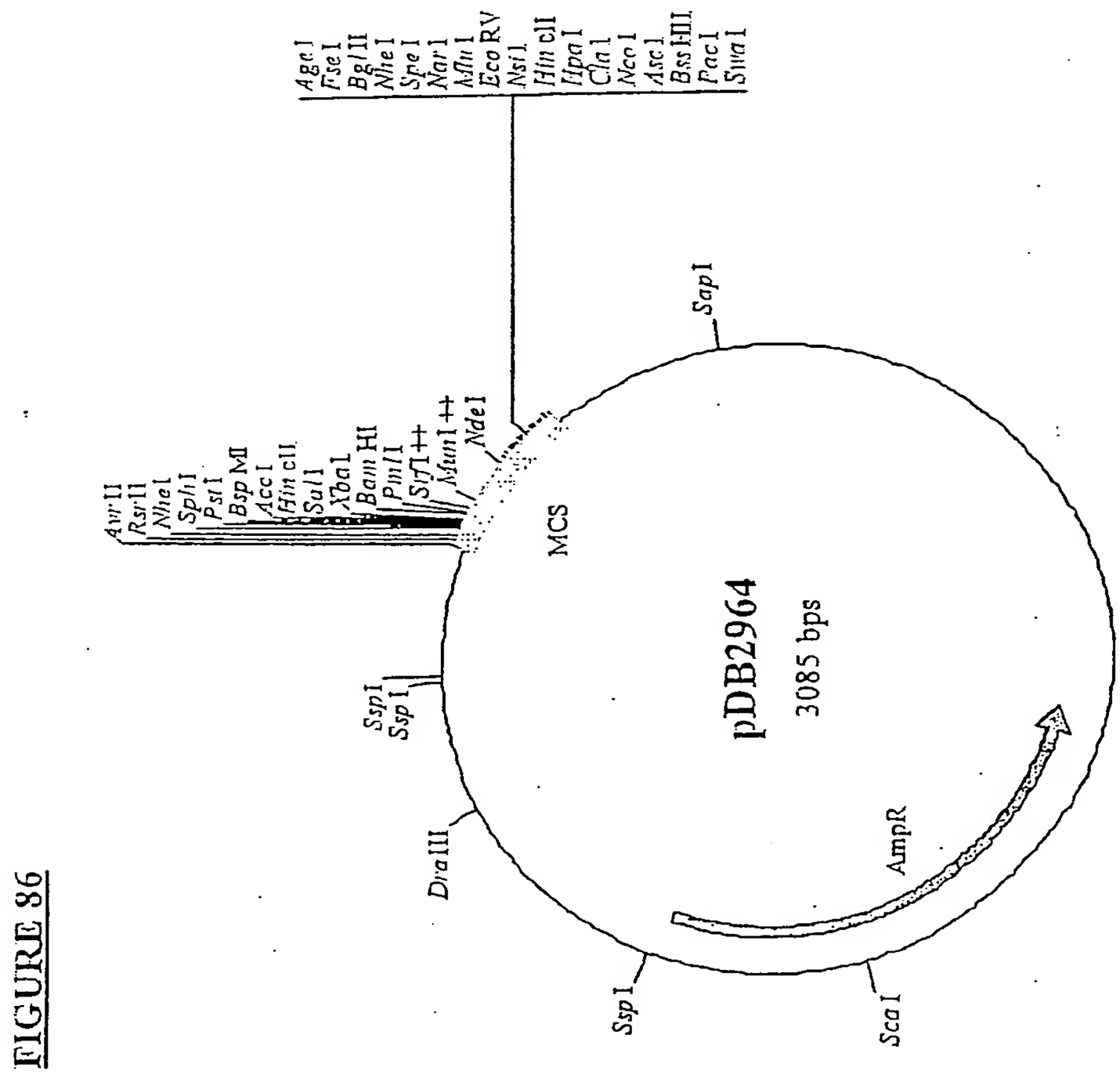


FIGURE 87

FIGURE 89

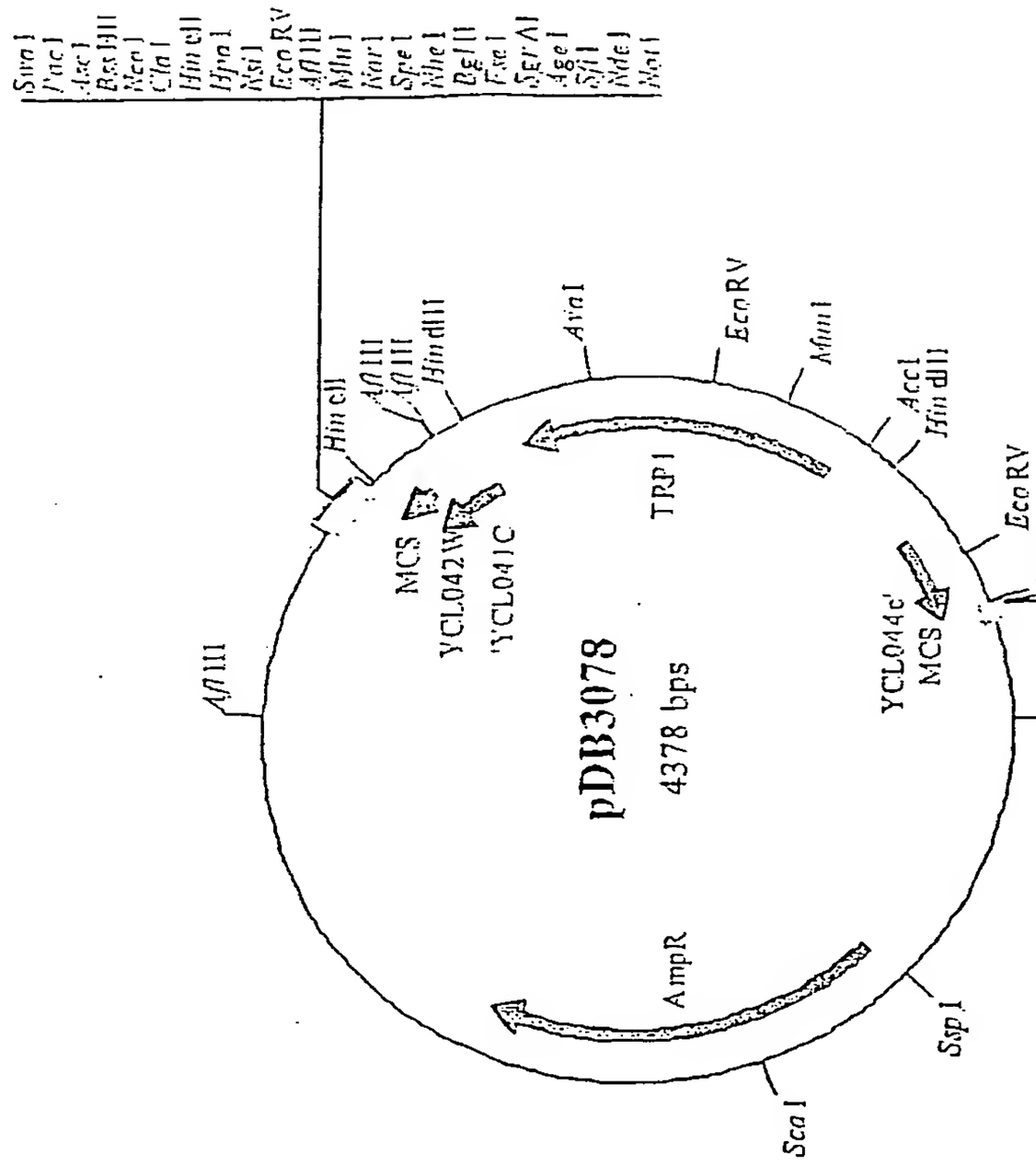


FIGURE 88

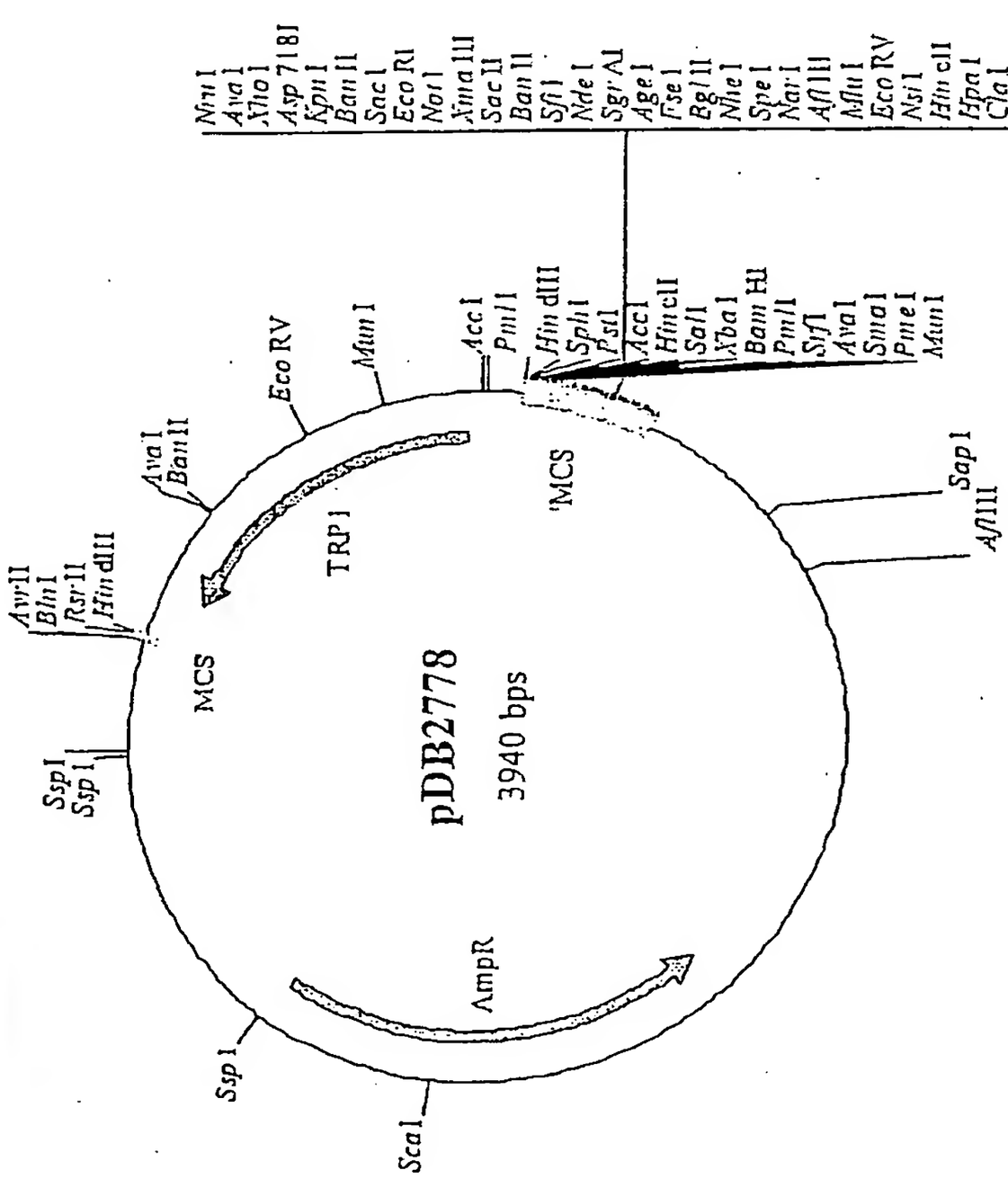


FIGURE 90

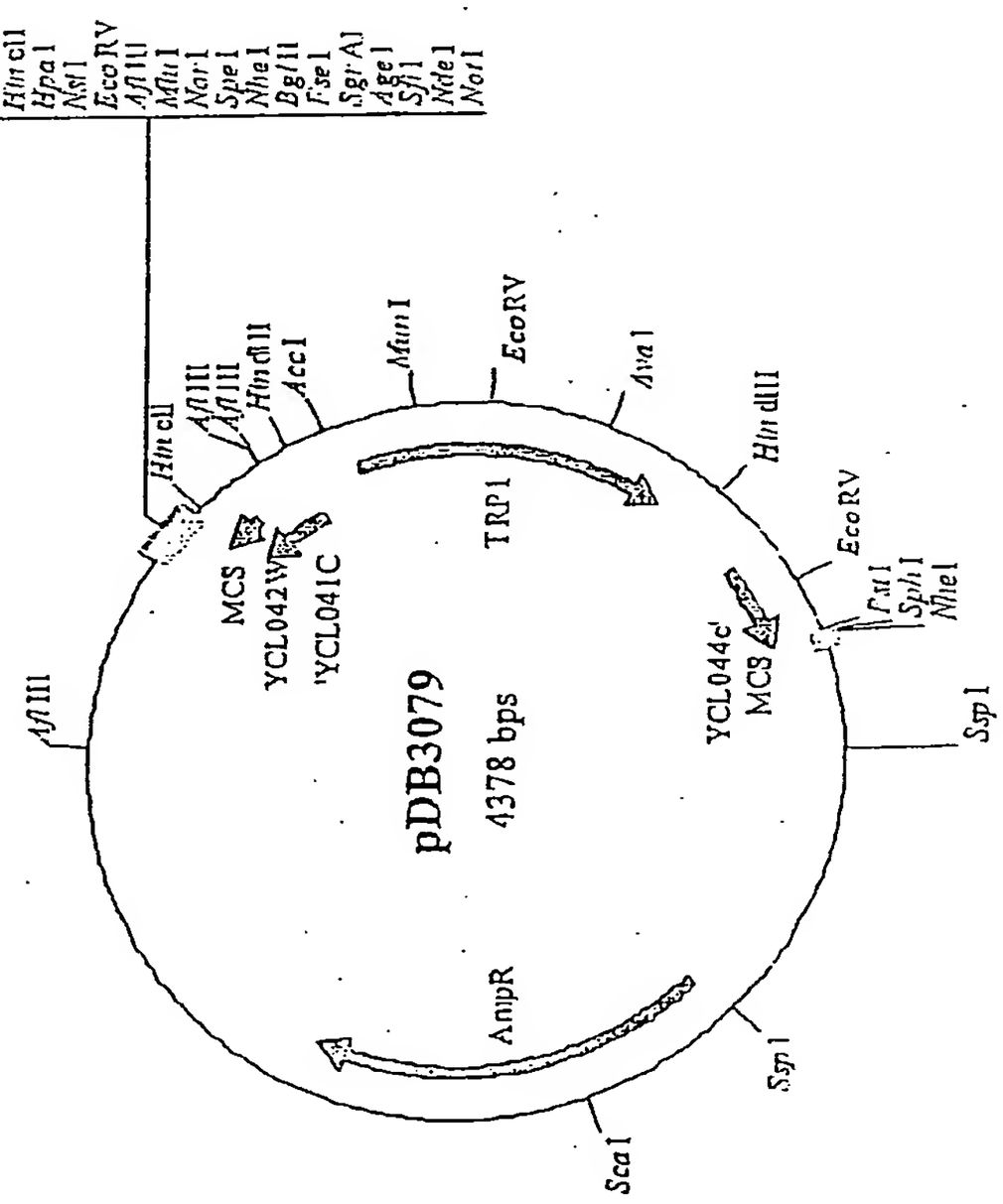
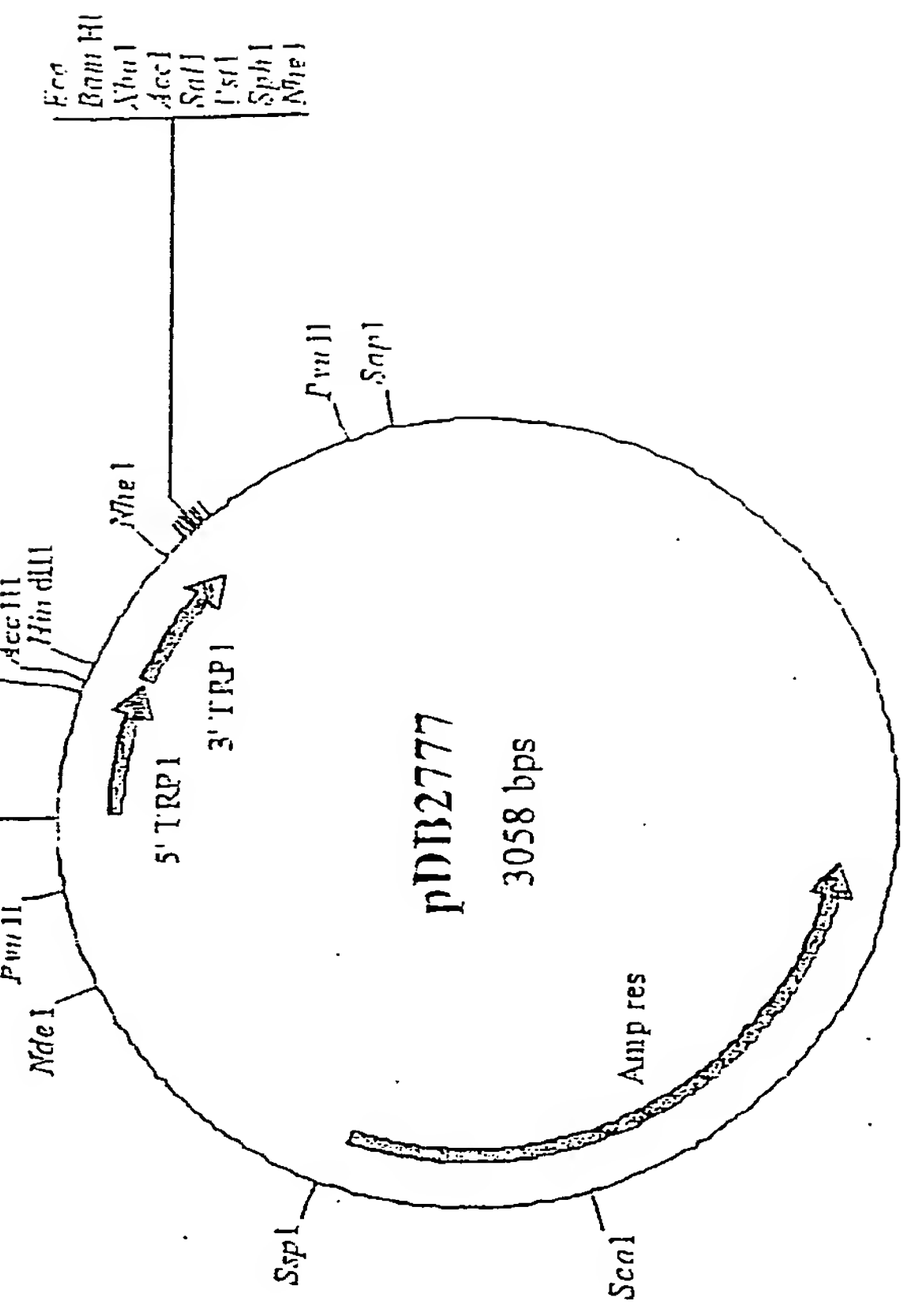


FIGURE 91



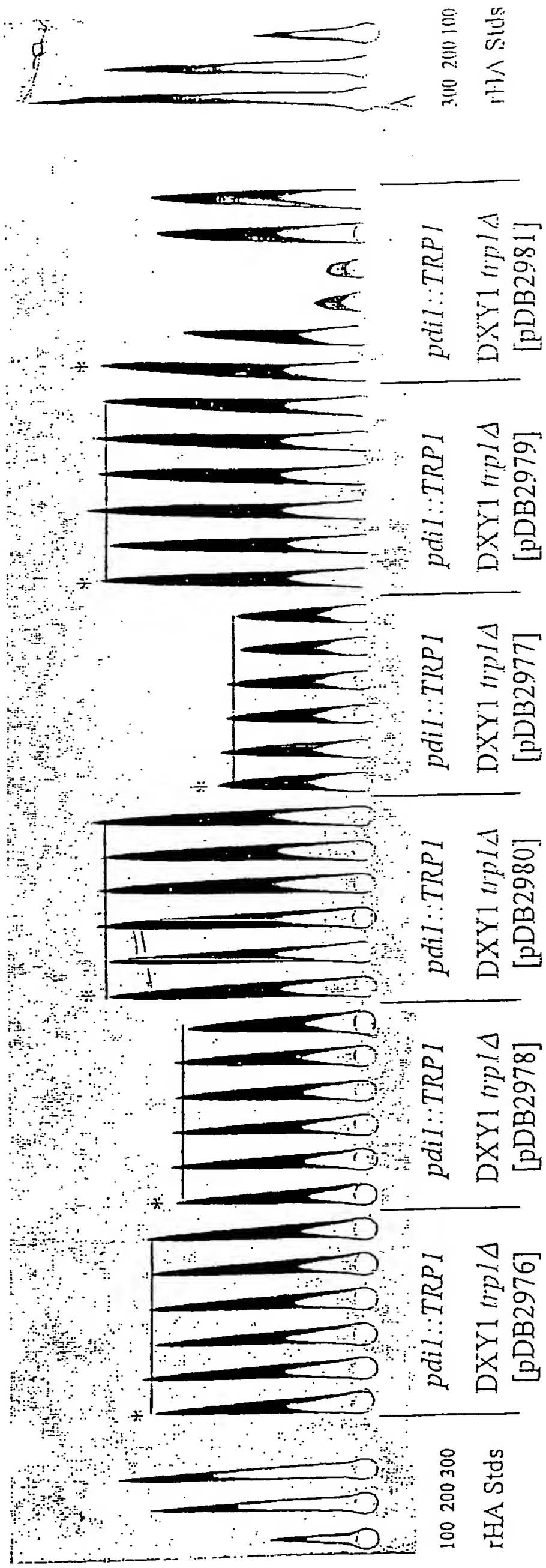

rHA Stds
300 200 100
pdi1::TRP1
DXY1 trp1Δ
[pDB2981]
pdi1::TRP1
DXY1 trp1Δ
[pDB2979]
pdi1::TRP1
DXY1 trp1Δ
[pDB2977]
pdi1::TRP1
DXY1 trp1Δ
[pDB2980]
pdi1::TRP1
DXY1 trp1Δ
[pDB2978]
pdi1::TRP1
DXY1 trp1Δ
[pDB2976]
rHA Stds
100 200 300

FIGURE 92

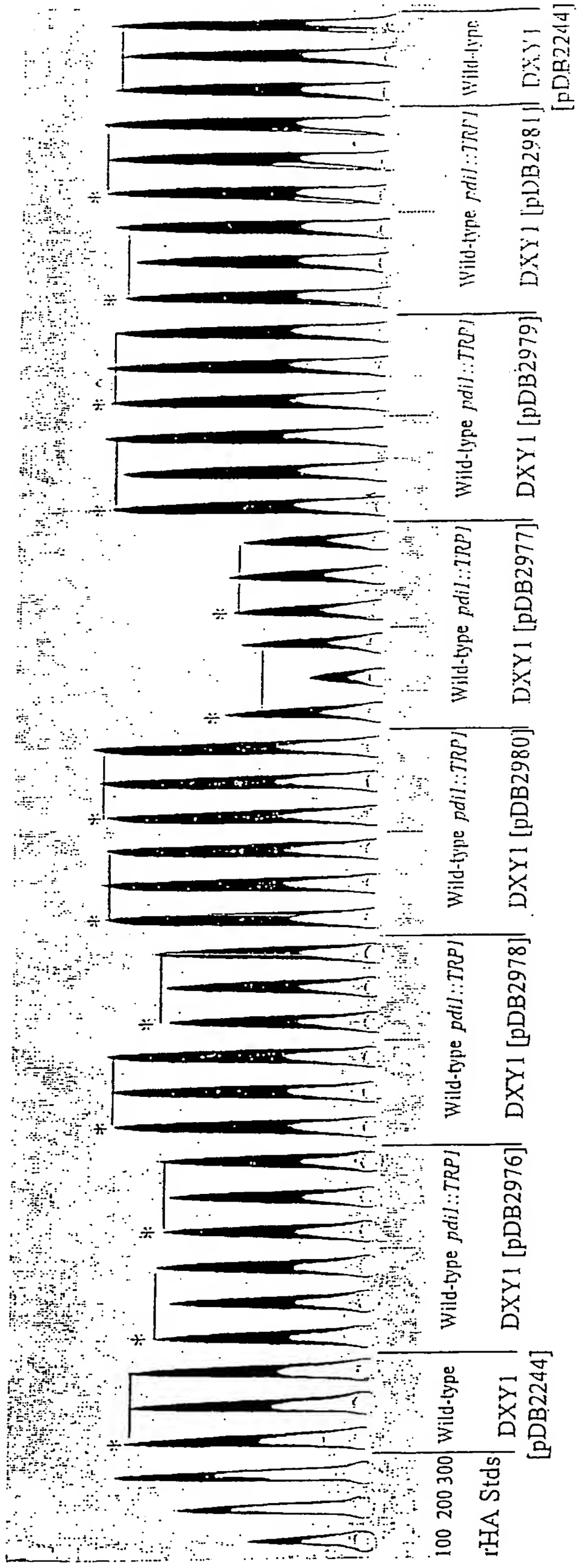

100 200 300
rHA Stds
Wild-type
DXY1
[pDB2244]
Wild-type pdi1::TRP1
DXY1 [pDB2976]
Wild-type pdi1::TRP1
DXY1 [pDB2977]
Wild-type pdi1::TRP1
DXY1 [pDB2980]
Wild-type pdi1::TRP1
DXY1 [pDB2978]
Wild-type pdi1::TRP1
DXY1 [pDB2981]
Wild-type
DXY1
[pDB2244]

FIGURE 93

Figure 94A

Alignment Workspace of ExL.meg.J. Hein (Weighted)
20 December 2004 15:04

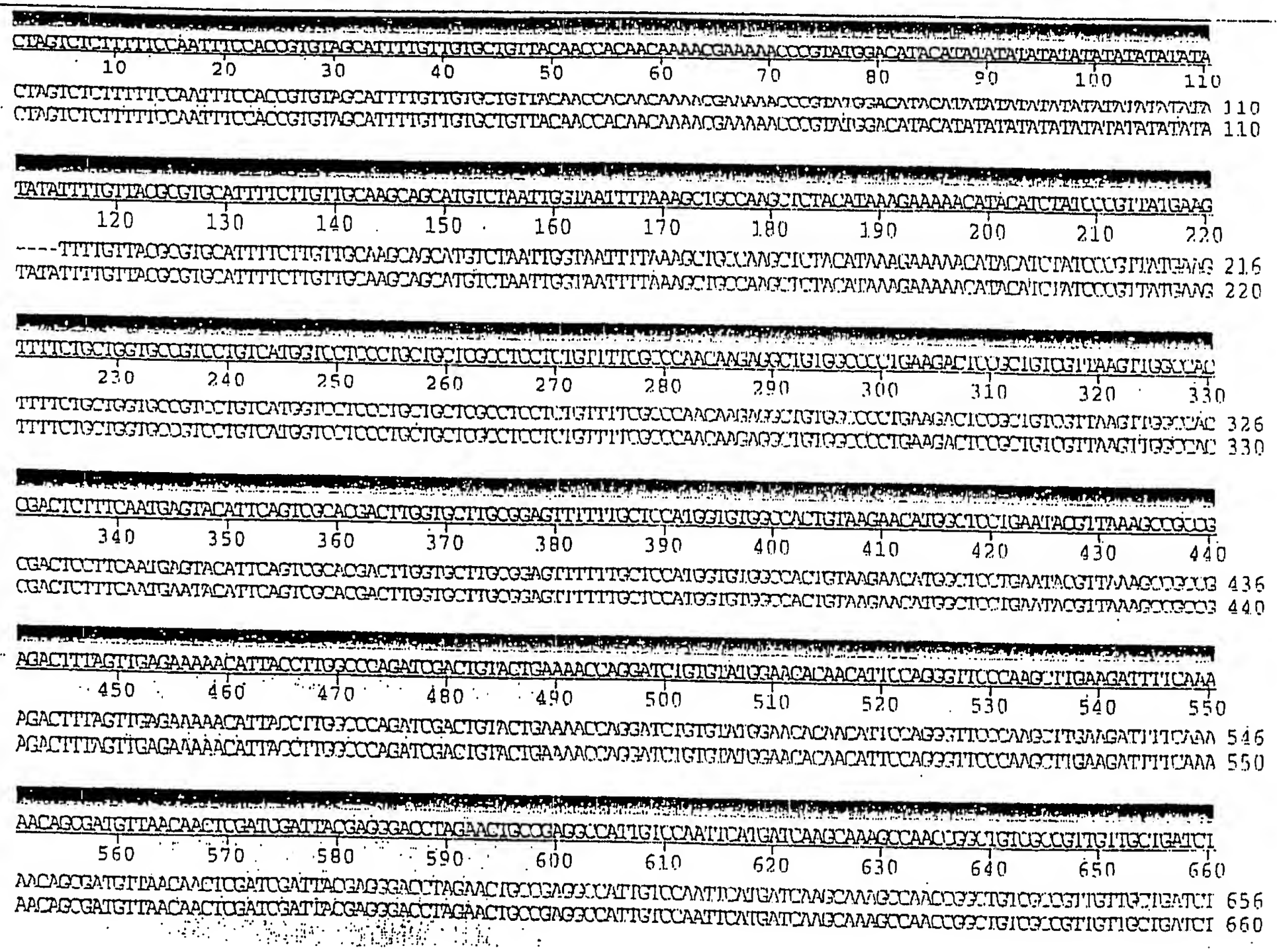


Figure 94B

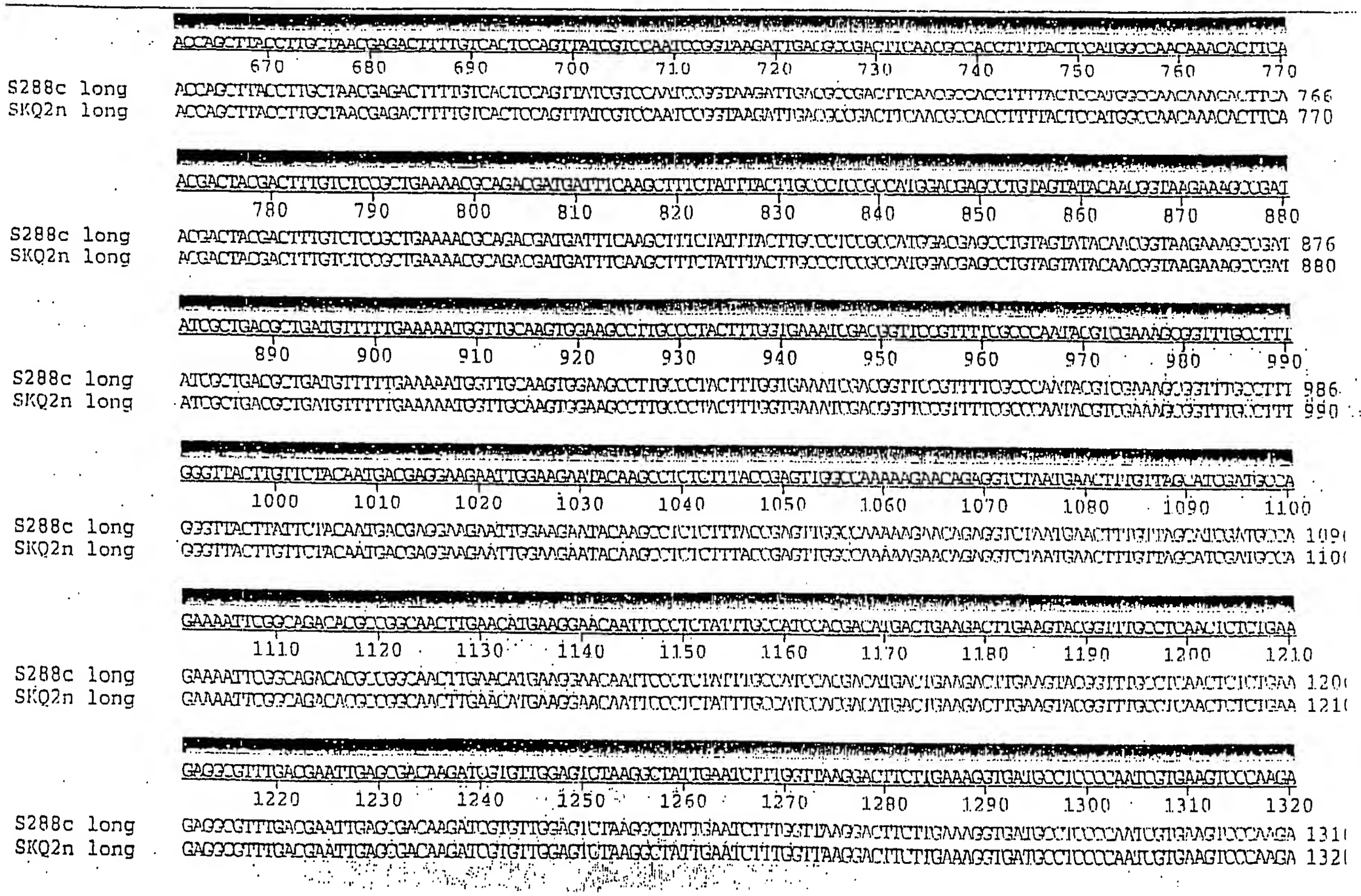
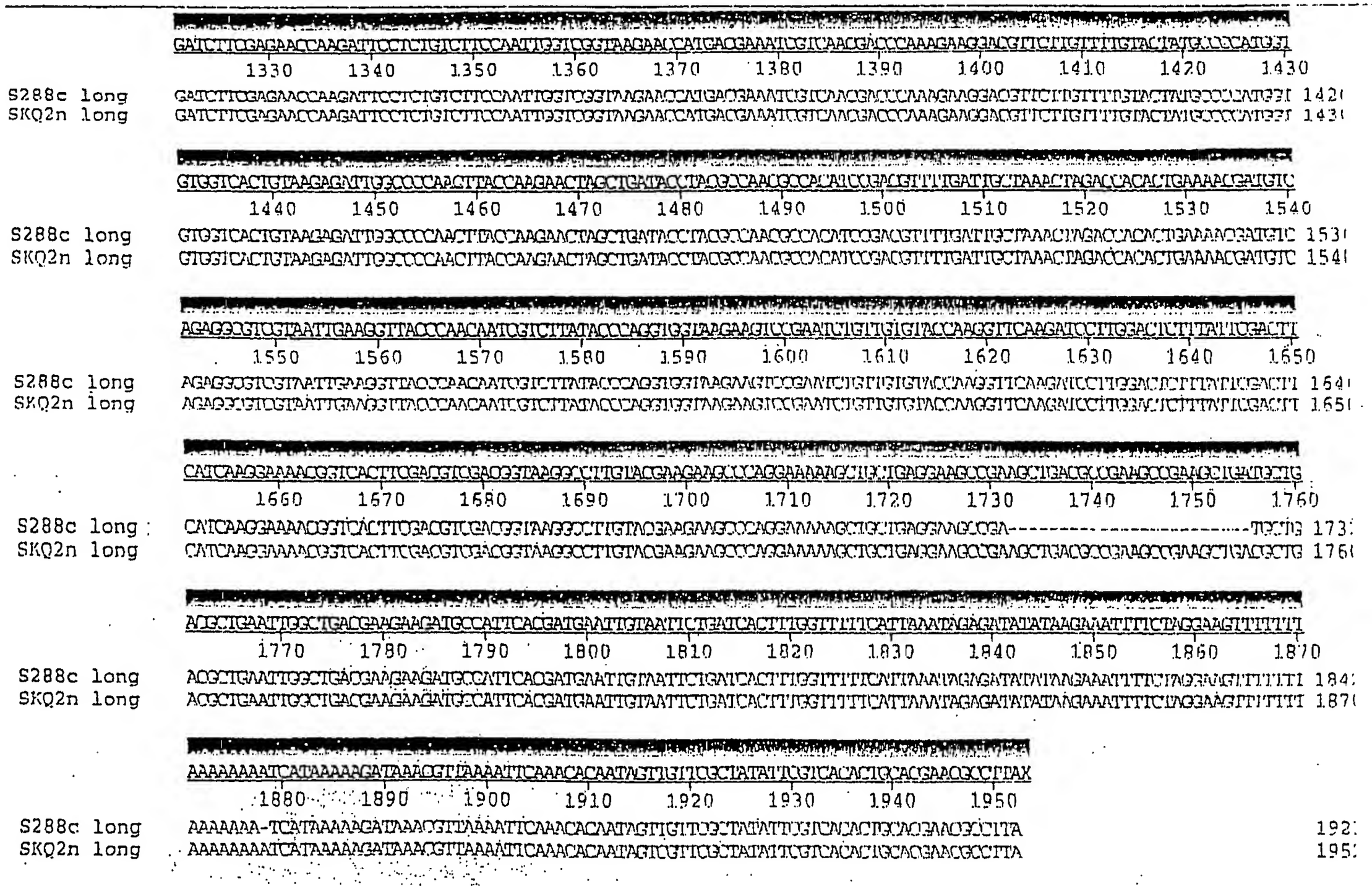

670 680 690 700 710 720 730 740 750 760 770
S288c long ACCAGCTTACCTTGCTAACGAGACTTTTGTCACTCCAGTTATCGTCCAATCCGGTAAGATTGACGCCGACTTCAACGCCACCTTTTACTCCATGGCCAACAAACACTTCA 766
SKQ2n long ACCAGCTTACCTTGCTAACGAGACTTTTGTCACTCCAGTTATCGTCCAATCCGGTAAGATTGACGCCGACTTCAACGCCACCTTTTACTCCATGGCCAACAAACACTTCA 770
780 790 800 810 820 830 840 850 860 870 880
S288c long ACGACTACGACTTTGTCTCCGCTGAAAACGCAGACGATGATTTCAAGCTTTCTATTTACTTGCCCTCCGCCATGGACGAGCCTGTAGTATACAACGGTAAGAAAGCCGAT 876
SKQ2n long ACGACTACGACTTTGTCTCCGCTGAAAACGCAGACGATGATTTCAAGCTTTCTATTTACTTGCCCTCCGCCATGGACGAGCCTGTAGTATACAACGGTAAGAAAGCCGAT 880
890 900 910 920 930 940 950 960 970 980 990
S288c long ATCGCTGACGCTGATGTTTTTGAAAAATGGTTGCAAGTGGAAGCCTTGCCCTACTTTGGTGAAATCGACGGTTCCGTTTTCGCCCAATACGTCGAAAGCGGTTTGCCTTT 986
SKQ2n long ATCGCTGACGCTGATGTTTTTGAAAAATGGTTGCAAGTGGAAGCCTTGCCCTACTTTGGTGAAATCGACGGTTCCGTTTTCGCCCAATACGTCGAAAGCGGTTTGCCTTT 990
1000 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
S288c long GGGTTACTTGTTCTACAATGACGAGGAAGAATTGGAAGAATACAAGCCTCTCTTTACCGAGTTGGCCAAAAAGAACAGAGGTCTAATGAACTTTGTTAGCATCGATGCCA 109
SKQ2n long GGGTTACTTGTTCTACAATGACGAGGAAGAATTGGAAGAATACAAGCCTCTCTTTACCGAGTTGGCCAAAAAGAACAGAGGTCTAATGAACTTTGTTAGCATCGATGCCA 110
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200 1210
S288c long GAAAATTCGGCAGACACGCTGGCAACTTGAACATGAAGGAACAATTCCCTCTATTTGCCATCCACGACATTGACTGAAGACTTGAAGTACGGTTTGCCTCAACTCTCTTGAA 120
SKQ2n long GAAAATTCGGCAGACACGCTGGCAACTTGAACATGAAGGAACAATTCCCTCTATTTGCCATCCACGACATTGACTGAAGACTTGAAGTACGGTTTGCCTCAACTCTCTTGAA 121
1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
S288c long GAGGCGTTTGACGAATTGAGCGACAAGATCGTGTTGGAGTCTAAGGCTATTGAATCTTTGGTTAAGGACTTCTTGAAAGGTGATGCCTCCCCAATCGTGAAGTCCCAAGA 131
SKQ2n long GAGGCGTTTGACGAATTGAGCGACAAGATCGTGTTGGAGTCTAAGGCTATTGAATCTTTGGTTAAGGACTTCTTGAAAGGTGATGCCTCCCCAATCGTGAAGTCCCAAGA 132

Figure 94C



PCT/GB2004/005462

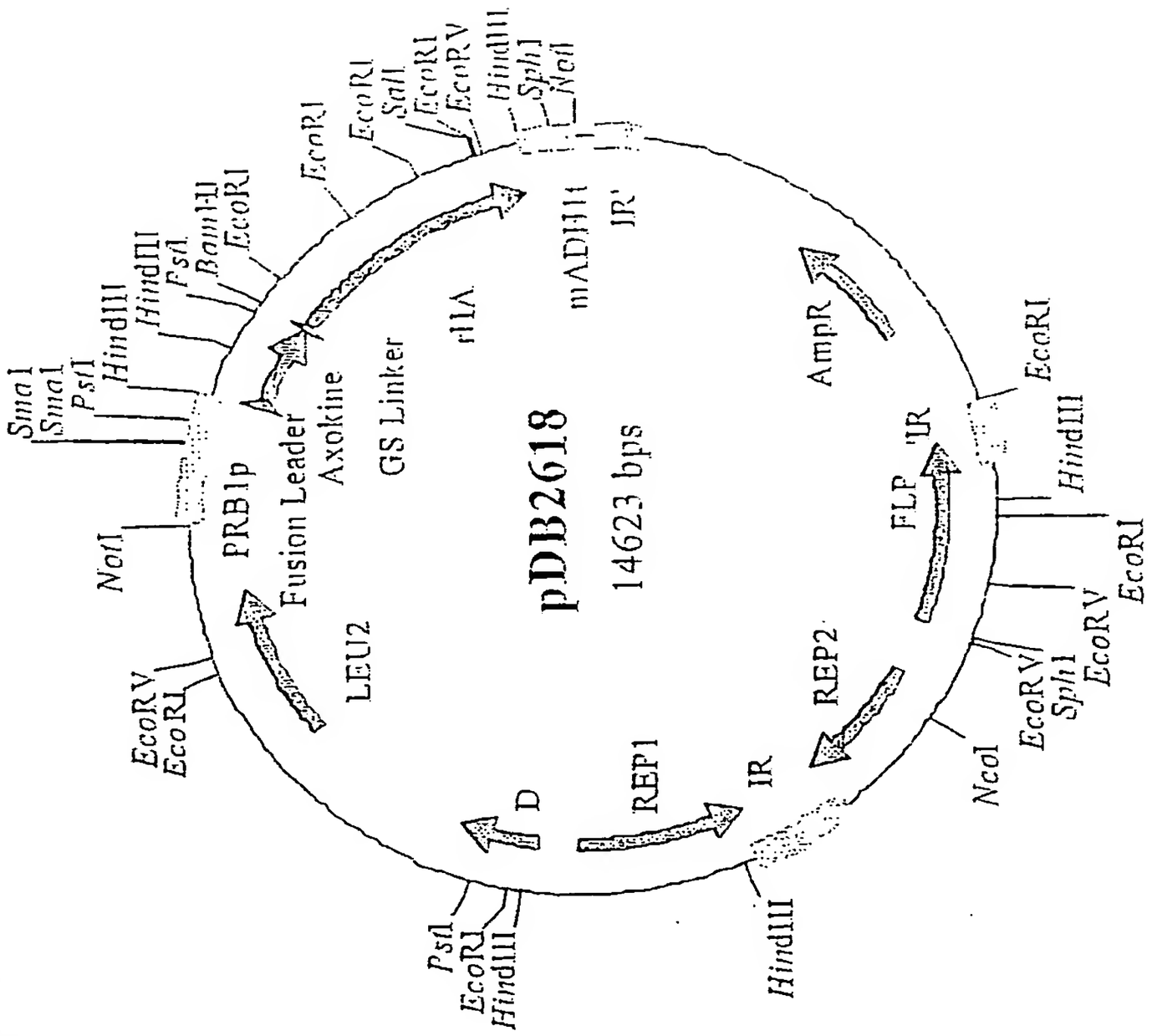


Figure 96

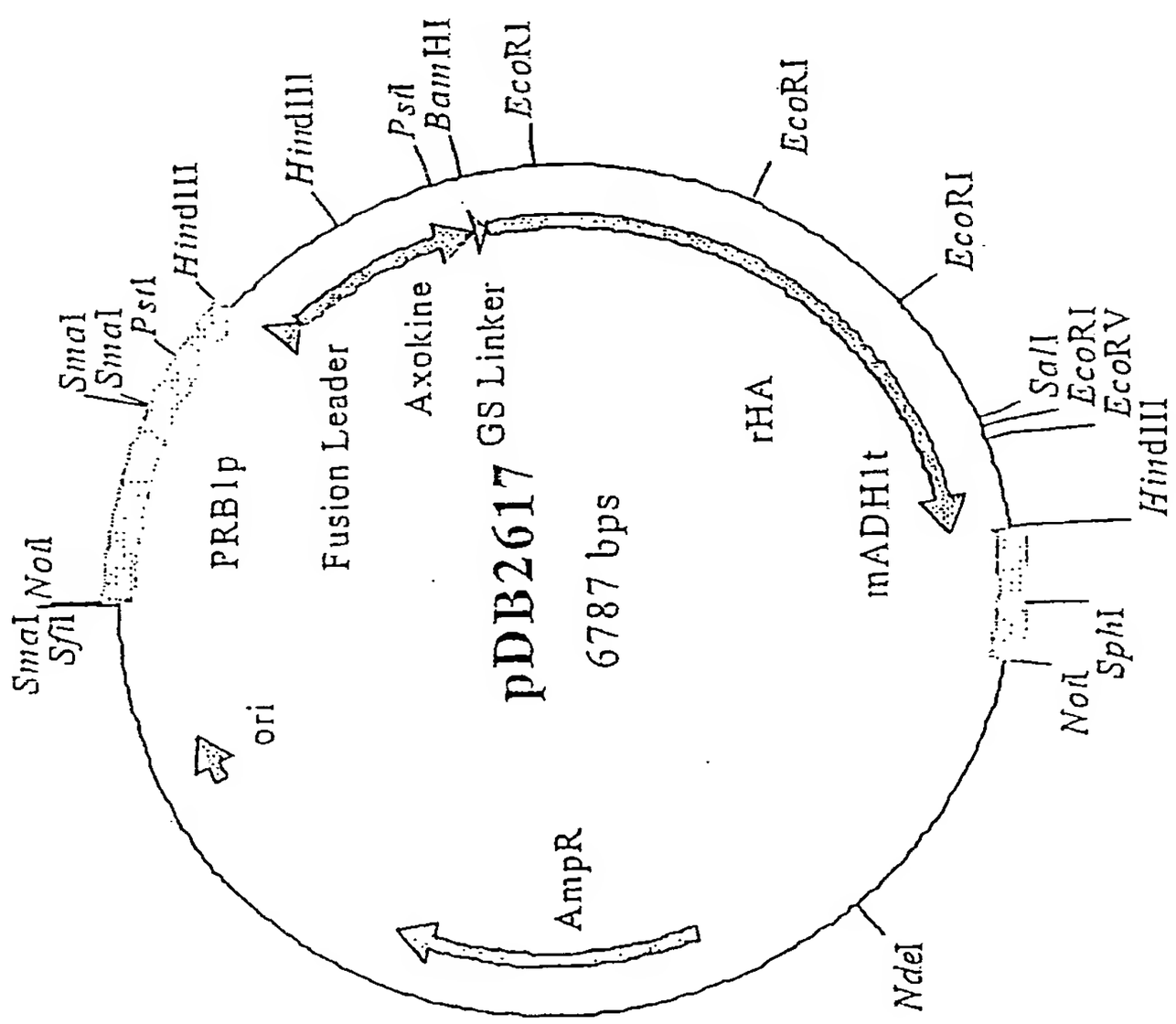


Figure 95